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ORIGINAL

MINUTES
OF THE
PHASE I WORKING GROUP
AND THE
BIOCHEMICAL MODULATORS ADVISORY GROUP

July 8-9, 1986

COMPLETED

Investigational Drug Branch
Cancer Therapy Evaluation Program
National Cancer Institute
Bethesda, Maryland

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AGENDA

BIOCHEMICAL MODULATORS ADVISORY GROUP
AND
PHASE I WORKING GROUP MEETINGS

NIH, Building 31, Conference Room 6
July 8-9, 1986

July 8--Morning Session

BIOCHEMICAL MODULATORS ADVISORY GROUP MEETING

8:15 a.m.	Introduction	Dr. R. Johnson SmithKline Beckman
8:20 a.m.	Cisplatin: Structural Chemistry and Molecular Biology	Dr. A. Pinto MIT
8:45 a.m.	Potential Mechanisms of Resistance to Cisplatin	Dr. A. Eastman Eppley Institute
9:10 a.m.	Platinum and Nucleosides: Interaction with 5-Fluorouracil	Dr. [illegible]. Scanlon City of Hope
9:35 a.m.	Platinum and Nucleosides: Studies with Ara-A	Dr. D. Spriggs DFCC
9:55 a.m.	Platinum and Nucleosides: Synergistic Interaction with Ara-C	Dr. B. Drewinko M.D. Anderson
10:15 a.m.	COFFEE BREAK	
10:30 a.m.	Depletion of Glutathione by a Mechanism of Cisplatin Modulation	Dr. C. Litterst NCI
10:50 a.m.	Search for Drugs that Synergize with Cisplatin	Dr. S. Howell UCSD
11:10 a.m.	Discussion	
11:20 a.m.	Recent Results of PALA + 5-FU in Pancreatic Cancer	Dr. B. Ardalan Kenneth Norris Jr. Cancer Hospital
11:35 a.m.	Phase I Study of PALA/Dipyridamole	Dr. M. Markman Memorial Sloan Kettering
12:05 p.m.	LUNCH	

July 8—Afternoon Session

PHASE I WORKING GROUP MEETING

FAA

1:05 p.m.	Introduction	Dr. P. O'Dwyer NCI
1:10 p.m.	Mechanism Studies	Dr. T. Corbett Wayne State Univ.
1:20 p.m.	Preclinical Pharmacologic Studies	Dr. D Zaharko NCI
1:30 p.m.	D X 1	Dr. M. Grever Ohio State
1:40 p.m.	D X 1	Ms. T. Melink UMCC
1:50 p.m.	Weekly Schedule	Dr. R. Knight NCI
2:00 p.m.	Clinical Studies — Weekly Schedules	Dr. D. Von Hoff Univ. of Texas San Antonio
2:10 p.m.	Pharmacokinetics	Dr. L. Malspeis Ohio State
2:20 p.m.	EORTC Phase I Trials	Dr. S. Kaye Univ. of Glasgow
2:30 p.m.	Discussion	
3:00 p.m.	COFFEE BREAK	

Acodazole

3:15 p.m.	Introduction	Dr. B. Foster NCI
3:20 p.m.	Bolus	Dr. R. Pazdur Wayne State Univ.
3:30 p.m.	Weekly	Dr. D. Trump Univ. of Wisconsin
3:40 p.m.	Discussion	

July 8--Afternoon Session (Continued)

Amonafide

3:50 p.m.	Introduction	Dr. G. Sarosy NCI
3:55 p.m.	Bolus D X 5; Pharmacokinetics	Dr. S. Legha Dr. R. Newman M.D. Anderson
4:05 p.m.	Clinical Studies and Pharmacokinetics	Dr. J. Leiby Dr. L. Malspeis Ohio State
4:15 p.m.	IV Bolus Q 4 Wks	Dr. D. Von Hoff Univ. of Texas San Antonio
4:25 p.m.	24-Hour CIV Infusion	Dr. R. Richardson Mayo Clinic
4:35 p.m.	Discussion	

Quality of Life

4:45 p.m.		Ms. T. Melink Univ. of Texas San Antonio
4:55 p.m.	Discussion	

PHASE I WORKING GROUP MEETING

July 9--Morning Session

Tumor Necrosis Factor

8:00 a.m.	Introduction	Dr. M. Roper NCI
8:05 a.m.	Pharmacokinetics	Dr. S. Sherwin Genentech
8:20 a.m.	IV Q 1 W X 8 Wks	Dr. D. Trump Univ. of Wisconsin
8:30 a.m.	IV Daily X 5 Q 3 W	Dr. J. Kovach Mayo
8:40 a.m.	Discussion	

Taxol

8:50 a.m.	Introduction	Dr. G. Sarosy NCI
8:55 a.m.	Clinical Studies	Dr. P. Wiernik Albert Einstein
9:05 a.m.	Discussion	

Didemnin B

9:15 a.m.	Introduction	Dr. H. Chun NCI
9:20 a.m.	Single Bolus	Dr. D. Von Hoff Univ. of Texas San Antonio
9:25 a.m.	D X 5	Dr. J. Stewart Dr. W. Tong VMCC
9:35 a.m.	Discussion	

Pibenzimol

9:45 a.m.	Introduction	Dr. H. Chun NCI
9:50 a.m.	5-Day CI	Dr. E. Kraut Ohio State

PHASE I WORKING GROUP MEETING

July 9--Morning Session (Continued)

Pibenzimol (Continued)

9:55 a.m.	5-Day CI	Dr. L. Kvols or Dr. J. Edmonson Mayo Clinic
10:00 a.m.	Discussion	

Merbarone

10:10 a.m.	Introduction	Dr. H. Chun NCI
10:15 a.m.	5-Day CI	Dr. E. Kraut Dr. L. Malspeis Ohio State Univ.
10:25 a.m.	5-Day CI	Dr. R. Warrell MSK-I
10:30 a.m.	Discussion	
10:45 a.m.	COFFEE BREAK	

HMBA

11:00 a.m.	Introduction	Dr. H. Chun NCI
11:05 a.m.	10-Day CI	Dr. M. Fanucchi MSK-I
11:10 a.m.	10-Day CI	Dr. D. Ettinger Johns Hopkins
11:15 a.m.	Discussion	

Trimetrexate

11:25 a.m.	Introduction	Dr. P. O'Dwyer NCI
11:30 a.m.	Daily X 5	Dr. J. Stewart VRCC
11:40 a.m.	Daily X 5	Dr. L. Grochow Johns Hopkins

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PHASE I WORKING GROUP MEETING (Continued)

July 9--Morning Session (Continued)

Trimetrexate (Continued)

11:50 a.m.	Discussion	
12:00 noon	LUNCH	

July 9--Afternoon Session

PHASE II/III DISCUSSION

SR2508: Future Development

1:00 p.m.	Introduction	Dr. G. Sarosy NCI
1:05 p.m.	Clinical Trials: Future Development	Dr. N. Coleman DFCC
1:15 p.m.	Clinical Trials: Future Development	Dr. F. Caruso Roberts Labs.
1:35 p.m.	Discussion	

Ifosfamide: Sarcoma and Testicular Trials

1:45 p.m.	Introduction	Dr. G. Sarosy NCI
1:50 p.m.	Clinical Trials: Adult Sarcoma	Dr. K. Antman DFCC
2:05 p.m.	Clinical Trials: Adult Sarcoma	Mr. D. Amato ECOG
2:20 p.m.	Clinical Trials: Pediatric Sarcoma	Dr. C. Pratt St. Judes
2:30 p.m.	Clinical Trials: Pediatric Sarcoma	Dr. J. Miser NCI
2:40 p.m.	Clinical Pharmacology: Pediatric Trials	Dr. M. Goren St. Judes
2:50 p.m.	Clinical Trials: Testicular Carcinoma	Dr. P. Loehrer Indianapolis
3:00 p.m.	Discussion	
3:15 p.m.	Adjournment	

BIOCHEMICAL MODULATORS ADVISORY GROUP

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POTENTIAL MECHANISMS OF RESISTANCE TO CISPLATIN

Alan Eastman

Eppley Institute for Research in Cancer, UNMC

The first clinical trial of nitrogen mustard

In 1942, a patient with a lymphosarcoma received "0.1 mg/kg compound X"

first course - marked reduction in tumor mass
second course - transient response
third course - ineffective

Important questions to be answered at the clinical level

1. Is resistance at the cellular level?

2. What are the patterns of resistance and cross-resistance in human tumors?

3. Does the spectrum of drugs influence development of resistance and cross-resistance?

Patterns of resistance to platinum complexes in experimental systems

Category	Selection Drug	Phenotype	Mechanism
IA	*cis*-DDP	cis-DDP^r DACH-Pt^s Melphalan^s Cd^s	?
IB	DACH-Pt	cis-DDP^s DACH-Pt^r Melphalan^s Cd^s	?
II	Melphalan	cis-DDP^r DACH-Pt^r Melphalan^r Cd^s	Elevated Glutathione
III	Cd	cis-DDP^r DACH-Pt^r Melphalan^r Cd^r	Elevated Metallothionein

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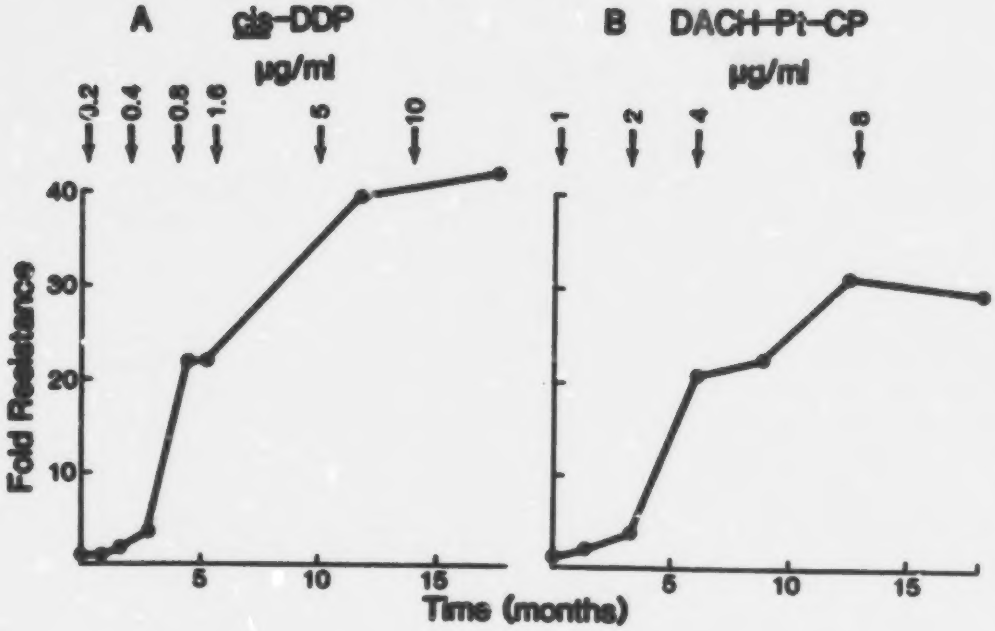

Table 1. Effects of various agents on the growth of cis-DDP-sensitive and -resistant L1210 cells*

Drug treatment	ID50 (μg/ml)		Fold resistance
	L1210/0	L1210/DDP	
cis-DDP (3 days)	0.04	1.25	31
cis-DDP (1 hr)	0.45	12	27
trans-DDP (3 days)	7	17	2.4
cis-DEP (3 days)	0.29	7.9	27
Melphalan (3 days)	0.17	0.33	1.9
Melphalan (1 hr)	1.3	1.8	1.4
Aziridinyl benzoquinone (3 days)	0.16	0.17	0
Neocarzinostatin (1 hr)	0.3	0.28	0
MNNG (3 days)	0.5	0.85	1.7
Methyl methanesulfonate (3 days)	7.5	9.25	1.2
Caffeine (3 days)	220	190	0
Ultraviolet irradiation (J/m²)	13	19.5	1.5

* Each value is the mean of at least two experiments with four determinations made at each dose in each experiment.

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TABLE 1.—Effects of various agents on the growth of sensitive and resistant L1210 cells

Drug treatment	ID50 (μg/ml)		
	L1210/0	L1210/DDP	L1210/DACH
cis-DDP	0.12	7.12 (60)*	0.37 (3.1)
trans-DDP	11.20	28.00 (2.4)	19.00 (1.7)
DACH-Pt-carboxyphthalate	0.46	0.65 (1.4)	13.00 (29)
DACH-Pt-SO$_4$	0.33	1.17 (3.5)	10.30 (31)
DACH-Pt-ascorbate	1.10	2.86 (2.6)	44.00 (40)
Melphalan	0.33	2.23 (6.8)	0.79 (2.4)

* Values in parentheses represent -fold resistance.

MECHANISMS OF RESISTANCE: DNA target

Decreased uptake
Increased efflux
Intracellular inactivation
Reduced reaction with DNA
Altered reaction with DNA
Enhanced DNA repair
Enhanced tolerance of DNA damage

Table 3. Properties of Pt-Sensitive and -Resistant L1210 cells and Hybrids

Cell Line	Chomosome modal value (range)	ID_{50} (ug/ml)	
		cis-DDP	DACH-Pt-SO_4
L1210/TGO	40 (38-42)	0.12	0.22
L1210/DDP_5	38 (37-40)	5.80 (48)[a]	1.40 (6.4)
TGO:DDP_5	74 (67-81)	1.55 (12)	0.73 (3.3)
L1210/DDP_{10}	38 (34-44)	13.20 (110)	3.92 (18)
TGO:DDP_{10}	70 (63-80)	1.90 (15)	1.32 (6.0)
L1210/DACH	39 (37-40)	0.70 (5.8)	7.20 (33)
TGO:DACH	74 (67-84)	0.60 (5.0)	0.98 (4.4)

a. Values in parentheses represent fold resistance.

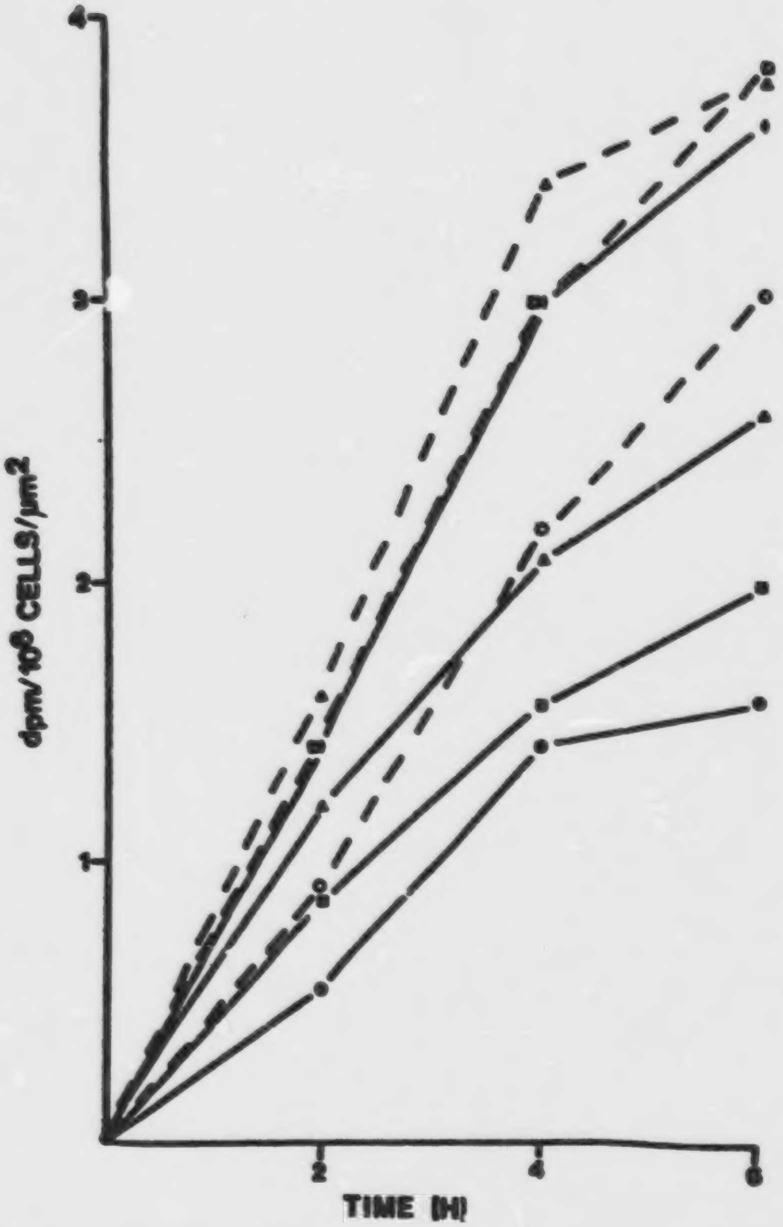

Figure 13. Time dependent accumulation of [^{3}H]-*cis*-DEP in cells and hybrids normalized to surface area. Cells (1×10^6) were incubated with 0.1 ug/ml of [^{3}H]-*cis*-DEP. At 0, 2, 4 and 6 h cell associated radioactivity was determined in L1210/0 (♦), L1210/DDP_5 (■), 0:DDP_5 (□), L1210/DDP_{10} (●), 0:DDP_{10} (○), L1210/DACH (▲) and 0:DACH (△).

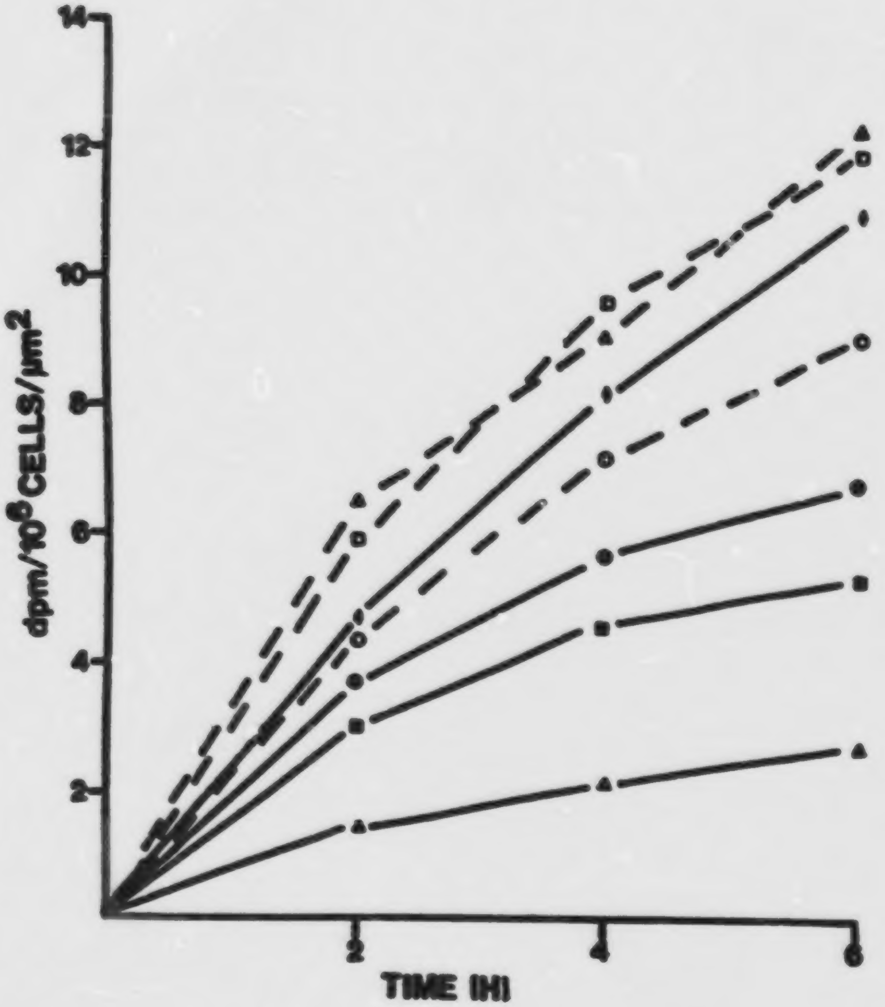

Figure 16. Time dependent accumulation of $[^3H]$-DACH-Pt-CL_2 in L1210 cells and hybrids normalized to surface area. Cells (1×10^6) were incubated with 0.31 mg/ml $[^3H]$-DACH-Pt-Cl_2. At 0, 2, 4 and 6 h the cell associated radioactivity was determined in L1210/0 (♦), L1210/DDP_5 (■), 0:DDP_5 (□), L1210/DDP_{10} (●), 0:DDP_{10} (○), L1210/DACH (▲) and 0:DACH (△) cells or hybrids.

Cell Line	GSH (µg/mg protein) - BSO	GSH (µg/mg protein) + BSO	*cis*-DDP ID_{50} (µg/ml) - BSO	*cis*-DDP ID_{50} (µg/ml) + BSO
L1210/0	6.2	2.9	0.48	0.45
L1210/DDP	12.1	5.8	16.2	16.8

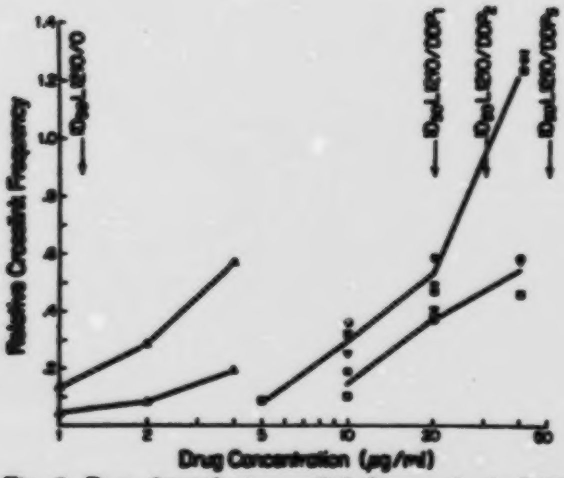

Fig. 5. Dose dependent cross-link frequencies in L1210 cells. Cells were treated with indicated concentrations of *cis*-DDP for 1 h. Posttreatment incubations were for 12 h to allow for maximum cross-link effect. Relative cross-link frequencies were determined after alkaline elution for the cell lines L1210/0 (▲, △), L1210/DDP$_1$ (■, □), L1210/DDP$_2$ (●, ○) and L1210/DDP$_3$ (▼, ▽). Total cross-links (▲, ■, ●, ▼) and proteinase resistant cross-links (△, □, ○, ▽) are shown.

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cis-DDP adducts in DNA

Monofunctional: G-Pt

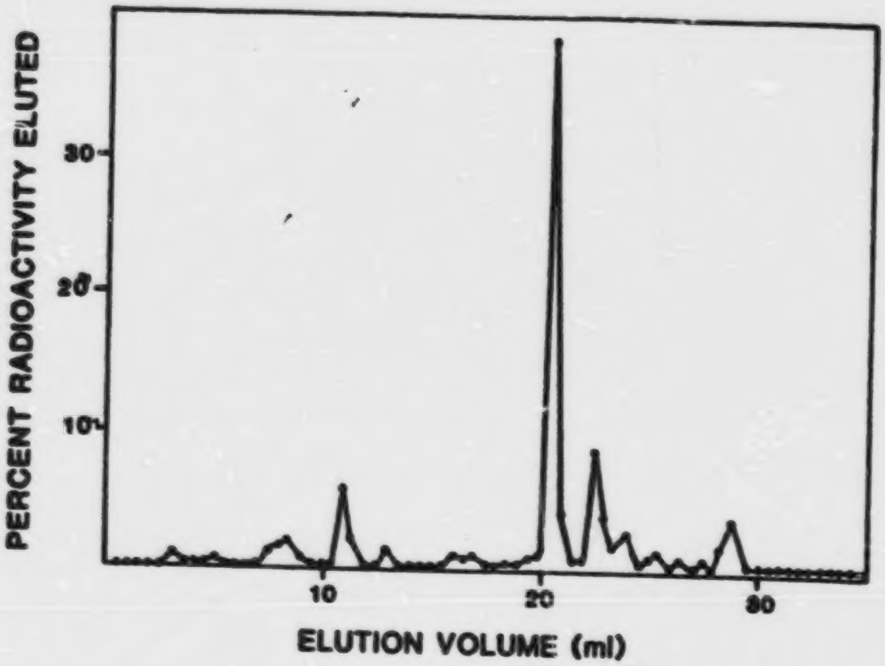
Intrastrand: $\widehat{GpG}$ $\widehat{ApG}$ $\widehat{GpNpG}$ $\widehat{ApNpG}$

Interstrand: G-Pt-G

Table III. The effect of incubation time on the percent of each adduct detected after reaction of [^{3}H]-*cis*-DEP with double-stranded DNA. All reactions were stopped by removal of unreacted drug and monofunctional adducts were trapped with thiourea (TU).

incubation time (min)	post-incubation time (hr)	percent radioactivity eluted			
		dG-Pt-TU	$d\widehat{Gpd}G$	$d\widehat{Apd}G$	dG-Pt-dG
15	0	42	32	2	9
30	0	40	36	3	8
60	0	26	45	5	9
60	1	14	54	9	9
60	2	4	57	15	9
60	16	0	60	18	6

HPLC SEPARATION OF [^{3}H]cis-DEP-DNA ADDUCTS IN L1210 CELLS



SUMMARY

1. cis-DDP and DACH-Pt resistant L1210 cells demonstrate multiple mechanisms of resistance.
2. Reduced accumulation is a recessive trait in hybrid cells.
3. Glutathione levels are increased in some Pt resistant cells. However, decreasing the intracellular glutathione levels does not increase sensitivity to cis-DDP.
4. A dominant mechanism operates subsequent to platination of DNA e.g. DNA repair or post replication repair.

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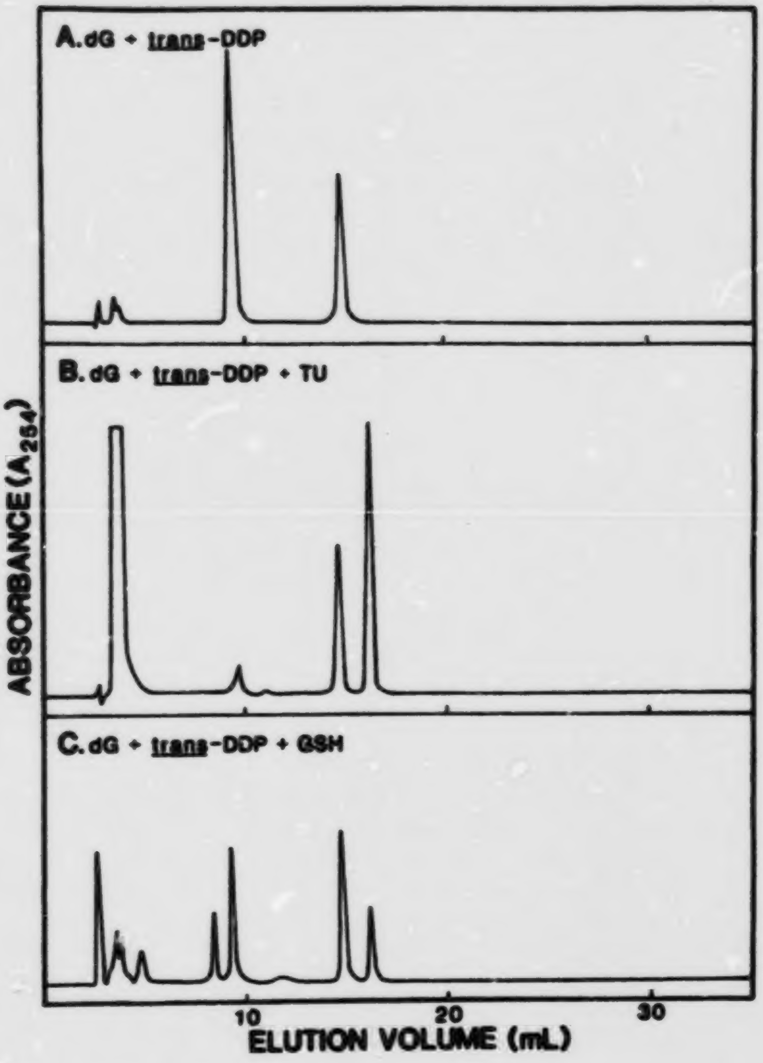
HPLC SEPARATION OF trans-DDP-dG STANDARDS



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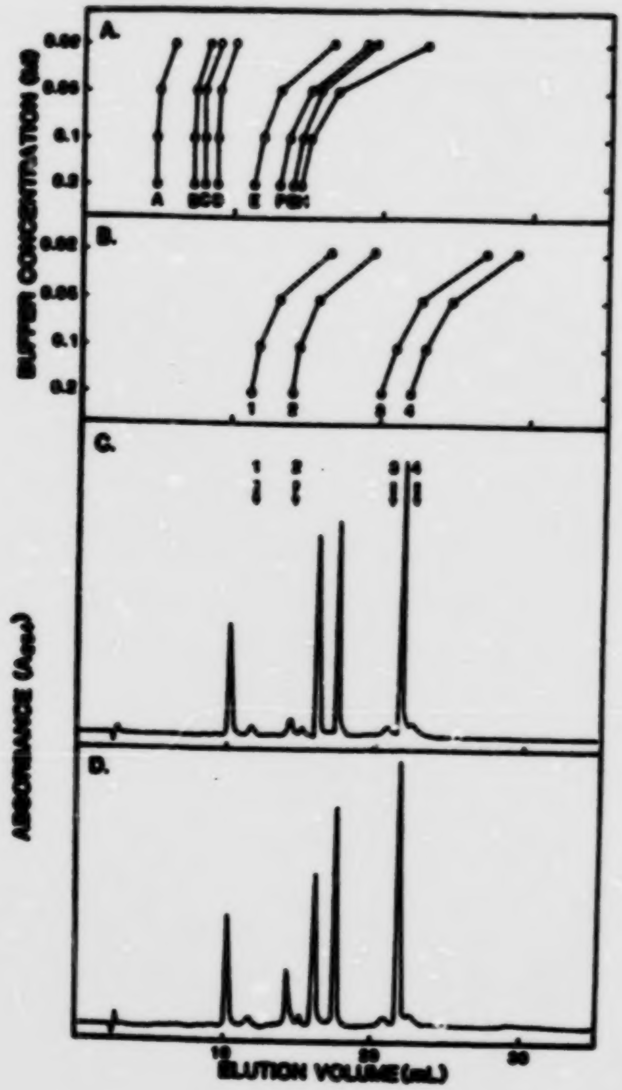

trans-DDP adducts in DNA

1. C-Pt-G
2. G-Pt-G
3. G-Pt-CpT
4. G-Pt-CpA

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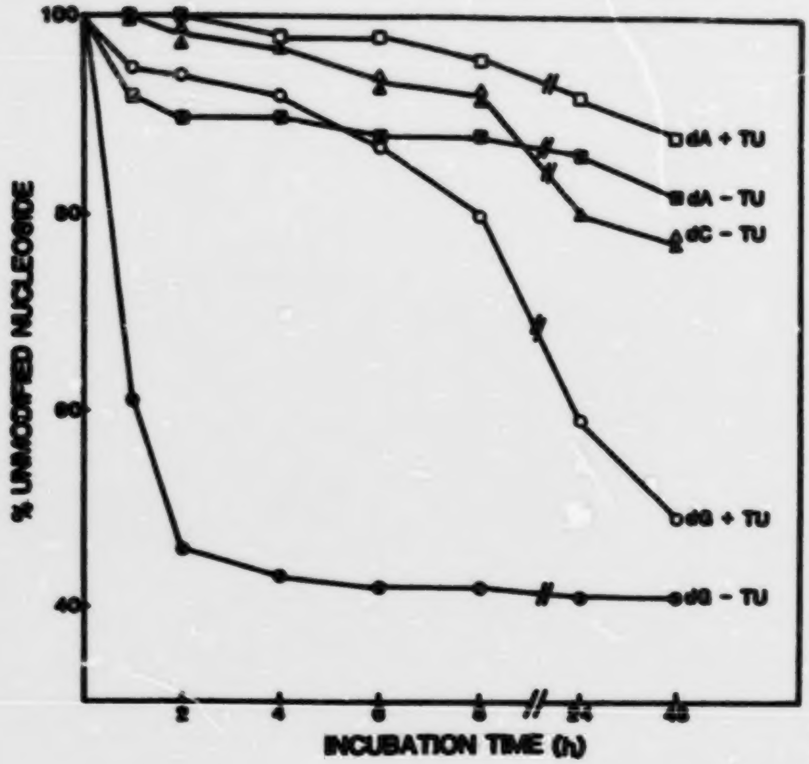

	GSH (μg/10^6 cells)		trans-DDP ID_{50} (μg/ml)	
Cell line	-BSO	+BSO	-BSO	+BSO
L1210/0	2.69	1.69	9.6	4.35
L1210/DDP	4.74	2.15	71	18.5

SUMMARY

1. trans-DDP produces a high level of monofunctional adducts.
2. Thiol agents such as thiourea and glutathione bind to and labilize trans-DDP monofunctional adducts.
3. Decreasing glutathione in cells sensitizes them to trans-DDP.
4. Trans-labilization may explain the apparent rapid repair of trans-DDP-DNA adducts.

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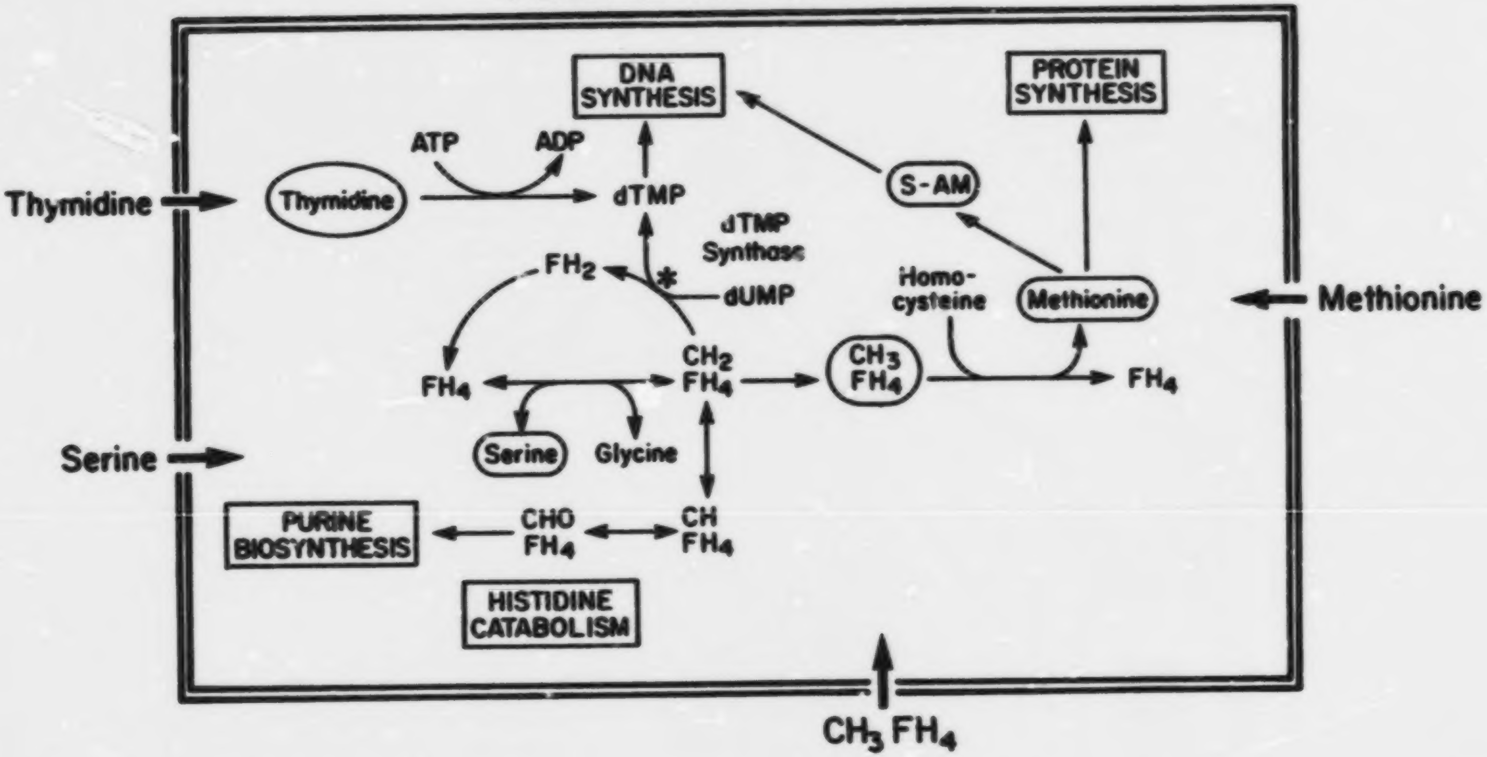
FOLATE METABOLISM



DNA SYNTHESIS
PROTEIN SYNTHESIS
ATP
ADP
Thymidine
Thymidine
dTMP
S-AM
dTMP Synthase
FH$_2$
*
dUMP
Homo-cysteine
Methionine
Methionine
CH$_2$ FH$_4$
CH$_3$ FH$_4$
FH$_4$
FH$_4$
Serine
Serine
Glycine
PURINE BIOSYNTHESIS
CHO FH$_4$
CH FH$_4$
HISTIDINE CATABOLISM
CH$_3$ FH$_4$

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TABLE 1
INHIBITION OF A2780 CELL GROWTH BY CISPLATINUM AND 5FUra

a.	Sequence	% Control Growth
1.	Cisplatinum (10uM) was added for 30 min. then 5FUra (5uM) added for 30 min.	24
2.	Cisplatinum (10uM) and 5FUra (5uM) were added together for 60 min.	32
3.	5FUra (5uM) was added for 30 min. then cisplatinum (10uM) added for 30 min.	41

b. Dose

Concentration (uM)			
Cisplatinum	5-FUra	Thd	% Control Growth
1	-	-	96
2	-	-	91
10	-	-	86
-	5	-	106
-	10	-	93
-	-	10	103
2	5	-	30
2	5	10	110
10	5	-	24
10	5	10	103

A2780 cells ($5x10^3$) were exposed to cisplatinum, dThd and/or 5-FUra under various conditions. The cells were pretreated for 30 min. with cisplatinum alone or with dThd, then 5-FUra was added for 30 min. The A2780 cells were exposed for a total of 60 min. to the drugs, then incubated for 7 days in drug-free medium. These experiments were performed in triplicate. The control cultures doubled 7 times during this period. The final cell number was 0.5-1.0 x 10^5 in untreated cultures (100%).

TABLE 2
DRUG PERMEABILITY IN A2780 CELLS

A. CISPLATINUM UPTAKE

(195M)Cisplatinum uptake into A2780 Cells (picomol/mg protein)

Time (min.)	Control	+5FUra
30	49.20	44.76
60	70.41	75.12
90	93.58	84.32

A2780 cells were exposed to 10uM of cisplatinum in Earle's balanced salt solution for 90 min. in the presence or absence of 5FUra (5uM). Cells were washed (4x) in ice cold PBS, lysed with NaOH (1N) at 24° overnight, then neutralized and counted as previously described (6).

B. 5FUra UPTAKE

(^{3}H)5FUra uptake into A2780 Cells (picomol/mg protein)

Time (min.)	Control	+Cisplatinum
60	13.95(±1.74)	14.4(±2.46)

A2780 cells were exposed to 5uM of (^{3}H)FUra in Earles balanced salt solution for 60 min. in the presence or absence of cisplatinum (10uM). Cells were washed (4x) in ice cold PBS to stop the reaction and counted as for cisplatinum uptake.

TABLE 3
dTMP SYNTHASE ACTIVITY IN THE PRESENCE AND ABSENCE OF CISPLATINUM

Conditions	dTMP Synthase Activity (picomol/min/10^7 cells)
- cisplatinum	170 (± 6)
+ cisplatinum	170 (± 23)

dTMP synthase activity was measured from the freshly prepared 12,000g supernatant fraction of A2780 cells (2-4 x 10^7) using the (^{3}H) release assay as previously described (12). Where indicated, cisplatinum (10uM) was pretreated for 15 min at 37° prior to the initiation of the reaction.

TABLE 4
(^{3}H)FdUMP BINDING ASSAY IN A2780 CELL SUPERNATANTS

Conditions	(^{3}H)FdUMP Binding Sites
Methylene-Tetrahydrofolate	(picomol/10^7 cells)
-	0.42(±0.02)
+	1.06(±0.05)

The number of FdUMP binding sites was determined in the freshly prepared 12,000g supernatant fraction from A2780 cells as previously described (13). Methylenetetrahydrofolate (250 umol)was added to the 12,000g supernatant for maximum FdUMP binding.

TABLE 5
CHANGES IN THE FOLATE POOLS AFTER TREATMENT WITH CISPLATINUM IN A2780 CELLS

Conditions	Methylene Tetrahydrofolate (picomol/10^7 cells)	Tetrahydrofolate (picomol/10^7 cells)
Control	3.36	2.68
Cisplatinum	5.06	4.18

A2780 cells (1-2 x 10^7 cells) were incubated at 37° for 30 min. in the presence or absence of cisplatinum (10uM). Cells were washed in ice cold PBS (2x) and aliquots were analyzed for folates as previously described (14).

TABLE 6
BINDING OF (^{3}H)FdUrd TO MACROMOLECULES IN A2780 CELLS

	*Treatment	Concentration (uM)	Net Acid-Soluble (^{3}H) Material (picomol/10^7 cells)
1.	Control	--	0.55
2.	Cisplatinum	10	1.36

*Cells were pretreated with cisplatinum for 30 min. prior to the addition of (^{3}H)FdUrd.

A2780 cells was treated with (^{3}H)FdUrd (5nM) for 90 min, washed with ice cold PBS (4x), counted and lysed with water. One aliquot of the lysate was used for protein determination, while a second was precipitated with TCA (5% v/v) and a third was incubated at 65° for 15 min. prior to acid precipitation. Acid-insoluble material was collected on Whatman glass filters. The net-acid soluble material was determined as the difference between acid-insoluble radioactivity with and without incubation at 65°.

TABLE 7

BINDING OF (^{3}H) FdUrd TO MACROMOLECULES IN PATIENT MALIGNANT ASCITES CELLS

Breast Carcinoma	Net Acid-Soluble (^{3}H) Material (picomol/10^{7} cells) Control	Cisplatinum*
1. Patient (A.H.)**	0.31	0.68
2. Patient (D.H.)**	0.22	0.47
3. Patient (G.M.)**	0.25	0.70

* Pretreated for 30 min. prior to the addition of (^{3}H) FdUrd

** Patient cells in culture for 7 days only.

A2780 cells was treated with (^{3}H) FdUrd (5nM) for 90 min. washed with ice cold PBS (4x), counted and lysed with water. One aliquot of the lysate was used for protein determination, while a second was precipitated with TCA (5%,v/v) and a third was incubated at 65° for 15 min. prior to acid precipitation. Acid-insoluble material was collected on Whatman glass filters. The net acid-soluble material was determined as the difference between acid-insoluble radioactivity with and without incubation at 65°.

CONCLUSIONS

1. Cisplatinum and 5-FUra are synergistic in the human ovarian carcinoma cell line, A2780. The pretreatment of cisplatinum prior to 5-FUra achieves maximum cytotoxicity.

2. Cisplatinum increases the 5,10 methylenetetrahydrofolate and tetrahydrofolate pools in A2780 cells.

3. Cisplatinum increases thymidylate synthase complex formation in intact cells.

4. Patient cells in vitro yield similar results with cisplatinum as seen with the A2780 cells.

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Amino Acid Membrane Transport Prosperties of L1210 Cells Resistant to Cisplatin

R.B. GROSS · K.J. SCANLON *

Summary

A mouse L1210 leukemia cells line was made 18-fold resistant to the chemotherapeutic agent cisplatin. The drug resistant cells exhibited changes in morphologic and biochemical properties from the sensitive cells. Uptake of labeled cisplatin was only slightly decreased in the resistant cells. However, the exogenous methionine requirement for these cells was 18-fold less than for parent cells. This decrease in the methionine requirement was also associated with differences in both methionine metabolism and transport. Membrane transport analysis revealed different K_m and Vmax properties for methionine and a nonmetabolizable amino acid analogue between the two cell lines. In L1210 cells resistant to cisplatin, decreases in amino acid transport, plus changes in amino acid substrate specificities, may be related to a drug-membrane phenomenon which could be one mechanism of resistance to chemotherapeutic agents.

INTRODUCTION

e/a

Cisplatin has been an effective anticancer drug, but its mechanism of action is still unclear[10,11]. Both the cis and trans isomers of platinum cross-link with the nucleotides of DNA but only the cis form has antitumor effects[9,11,14]. This would suggest that there may be other mechanisms influencing platinum-DNA interactions. Cisplatin[9,14] could exert an additional interaction at the plasma membrane as do other antineoplastic drugs[6]. The L1210 mouse leukemia cell line which is dependent on high concentrations of exogenous methionine for optimal growth[6] is a model system for screening chemotherapeutic agents and is also highly sensitive to cisplatin[12]. Therefore in L1210 cells, amino acid transport properties and optimal methionine requirements were studied to characterize the properties of cisplatin sensitive and resistant cells. There were significant differences in amino acid transport kinetics and substrate specificities between the cisplatin sensitive and resistant cells. The data suggest that membrane transport mechanisms and/or methionine requirements may be involved in the mechanism of action of cisplatin in L1210 cells. This work has previously been published as an abstract[2].

MATERIAL AND METHODS

Chemicals

L-(1-^{14}C) methionine (50 mCi/mmol) and L-(3-^{3}H) threonine (20 Ci/mmol) were purchased from Amersham, Arlington Heights, IL. Alpha (1-^{14}C) methyl-aminoisobutyric acid (51.6 mCi/mmol) (MeAIB) was purchased from New England Nuclear, Boston, MA. (^{195m}Pt) Cisplatin (145 mCi/mmol) was obtained from Oak

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Section of Biochemical Pharmacology, Department of Medical Oncology, City of Hope National Medical Center, Duarte, California 91010 USA.

* Kevin J. Scanlon is a Scholar of the leukemia Society of America and to whom reprint requests should be addressed.

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Ridge National Laboratories, Oak Ridge, TN [5]. Cisplatin (NSF = 119875-1) and transplatin (NSF = 131558-H) were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD.

Cell lines and growth media

The L1210 cells resistant to cisplatin (L1210DDP) were kindly supplied by Dr. Joseph Burchenal of Memorial-Sloan Kettering, New York. The L1210DDP cells were established from frozen mycoplasma-free stocks at three-month intervals. These cells had a stable resistance and were grown in the absence of drug. These resistant cells showed similar properties in morphology, generation time and cisplatin sensitivity to cells continually maintained in cisplatin. Stock cultures of L1210S [14] and L1210DDP cells were grown in Fischer's medium (GIBCO, Grand Island, NY) containing 100 mg/liter methionine (660 μM), 62.9 mg/liter penicillin and 100 mg/liter streptomycin supplemented with 10% horse serum [13]. The dose to inhibit 50% of the cell growth (ED$_{50}$) in soft agar was determined by continuously exposing exponentially growing L1210 cells to cisplatin or transplatin at concentrations of 0.1-50 μM. Controls (100% cell growth) were L1210S and L1210DDP cells grown in the absence of cisplatin. Cells grown in the presence of drug were compared to the controls [13].

Exogenous methionine requirements were determined by growing L1210S and L1210DDP cells in soft agar with methionine depleted Fischer's medium supplemented with 15% dialyzed horse serum and various concentrations of methionine (0-100 mg/ml). Experiments were performed in triplicate and the colonies were counted 10 days later. Optimal conditions for exponentially growing L1210 cells were characterized for Fischer's media with various concentrations of methionine in 15% dialyzed horse serum. The maximum growth at the optimal methionine concentration was then individually normalized to 100% for each cell line [13].

Uptake of labeled cisplatin

Lapeled cisplatin (195^m) had a specific activity of approximately 145 mCi/mmol, but due to its short half-life (4.02 days) the specific activity declined rapidly.

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uptake

salt

L1210S and L1210DDP cells was determined by incubating these cells (10^7 cells/ml) in Earle's balanced lt solution as previously described [14]. The cell pellet was washed three times with ice cold 0.9% NaCl, then dissolved in 0.5 ml NCS solution (Amersham, Arlington Heights, IL), solubilized overnight at 37° C and counted [14].

Methionine metabolism

L1210S and L1210DDP cells (5×10^6 cells/ml) were preincubated for 15 min in Fischer's media depleted of methionine and containing 15% dialyzed horse serum. (1-^{14}C)-methionine (500 μM) was then added to the cell incubation. The uptake of methionine and its metabolism into acid insoluble material was measured over 60 min as previously described [13].

Incubation media for amino acid transport studies

The incubation medium (IM-1) for determining Na-dependent amino acid transport was Earle's balanced salt solution (GIBCO). Incubation medium (IM-2) for determining Na-independent transport was Earle's balanced salt solution substituted with 116 mM choline chloride for 116 mM NaCl. The osmolality of IM-1 and IM-2 was 300 mOSM and the pH was 7.4 [14].

Transport measurements

Cells were harvested during logarithmic growth by centrifugation at 500 g, washed once with each specific incubation medium and resuspended to a final density of 5.0×10^6 cells/ml. Then labeled amino acid (methionine, methylaminoisobutyric acid or threonine) was added at the zero time point. At timed intervals, 200 μl samples were withdrawn and immediately separated from the incubation medium by centrifugation through a silicone-mineral oil gradient into 7% perchloric acid and counted as previously described [14]. The data presented in the charts was the mean of at least three separate experiments. The standard error was less than 10% of the mean for L1210DDP cells and less than 5% of the mean for L1210S cells. The kinetic parameters (K_t and V_{max}) were determined by iterative non-linear least squares program to the direct Michealis-Menton equation. The data were not weighted [13].

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Amino acid transport

When L1210S cells were preincubated with cisplatin, there was a decrease in methionine uptake (*Table 1*). Transplatin did not significantly influence the uptake of methionine in either the L1210S or the L1210DDP cells (*Table 1*).

Differences in the amount of initial Na-dependent and Na-independent transport between the L1210S and L1210DDP cells were noted. The amino acid substrates were examined over a range of concentrations. Initial Na-dependent transport for methionine, threonine, and MeAIB was lower in L1210DDP cells. The K_t for methionine uptake (*Figure 6*) for both cell lines was similar (253 µM), yet the V_{max} was decreased 3.17-fold in the DDP cells. The K_t for sodium dependent MeAIB uptake did not decrease in the DDP cells but the V_{max} decreased 4.5-fold (*Figure 7*). Threonine showed a 6-fold decrease in V_{max} in the DDP cells. There was also a change in the K_t for sodium dependent threonine transport (*Figure 8*).

Substrate competition experiments were used to characterize the different amino acid transport pathways. Labeled MeAIB sodium dependent uptake was blocked up to 70% by

TABLE 1 - *Inhibition of methionine transport by cis and transplatin.*

	Inhibition of Na-dependent Methionine Uptake (%)	
	L1210S	L1210DDP
Cisplatin (10 µM)	38.5 (±10)	5.0 (±2)
Transplatin (10 µM)	7.5 (±2)	5.0 (±2)

The Na-dependent uptake of methionine (500 µM) was measured in the presence and absence of cisplatin or transplatin. The drug (10 µM) was preincubated with the cells for 10 min at 37°. Th initial uptake of methionine was 5.5 and 2.8 pmoles/10^9 cells/min for L1210S and L1210DDP, respectively.

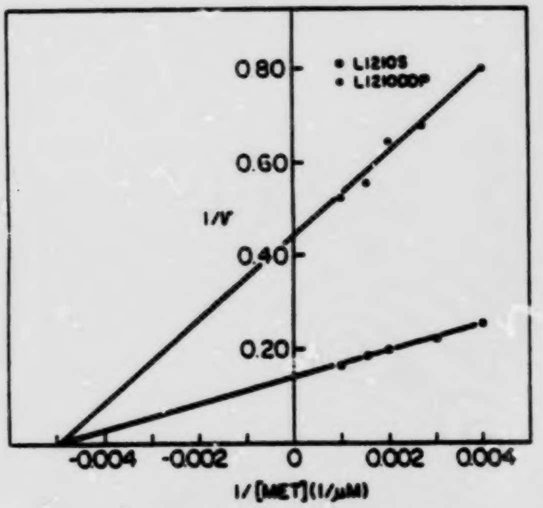

Figure 6 - *Total methionine uptake in L1210S and L1210DDP cells.* The total (1.0 min) uptake of methionine at various concentrations (250-1200 µM) was measured in L1210S (o) and L1210DDP (●) cells. 1/v was expressed as pmol/10^9 cells/min.

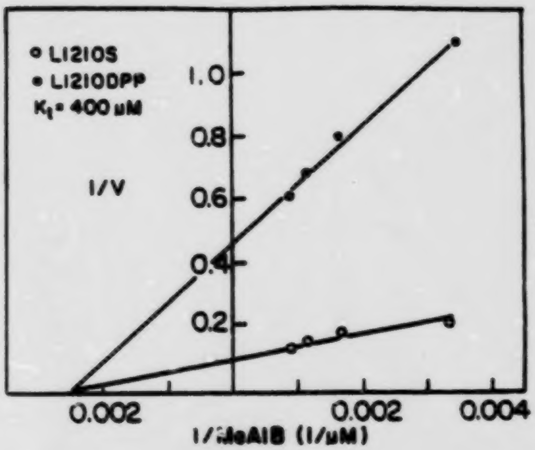

Figure 7 - *Initial MeAIB uptake in L1210S and L1210DDP cells.* Sodium dependent initial uptake (3 min) of MeAIB at various concentrations (250-1200 µM) was measured in L1210S (o) and L1210DDP (●) cells. 1/v was expressed as pmol/10^9 cells/min.

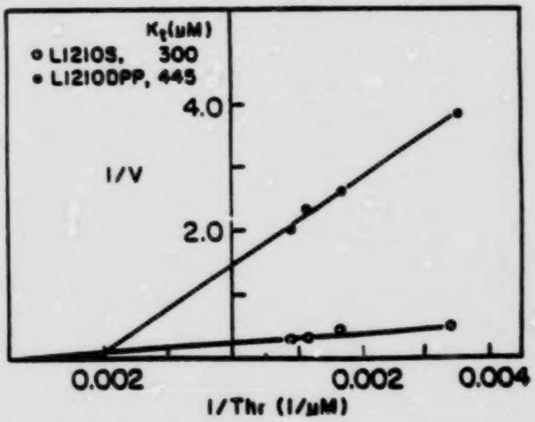

Figure 8 - *Threonine uptake in L1210S and L1210DDP cells.* Sodium dependent (1.5 min) uptake of threonine at various concentrations (250-1200 µM) was measured in L1210S (o) and L1210DDP (●) cells. 1/v was expressed as pmol/10^9 cells/min.

L1210DDP cells; however~~ver~~, L1210DDP cells metabolized two-fold more methionine into acid insoluble material. Therefore, the methionine transport to methionine metabolism ratio in L1210DDP of 1.5:1 was closer to unity than the 7:1 ratio in L1210S. Similar results for methionine transport and metabolism were obtained if the protein concentration was standardized instead of the cell number. The radioactivity in the acid soluble fraction remained as methionine as analyzed by paper chromatography (data not shown).

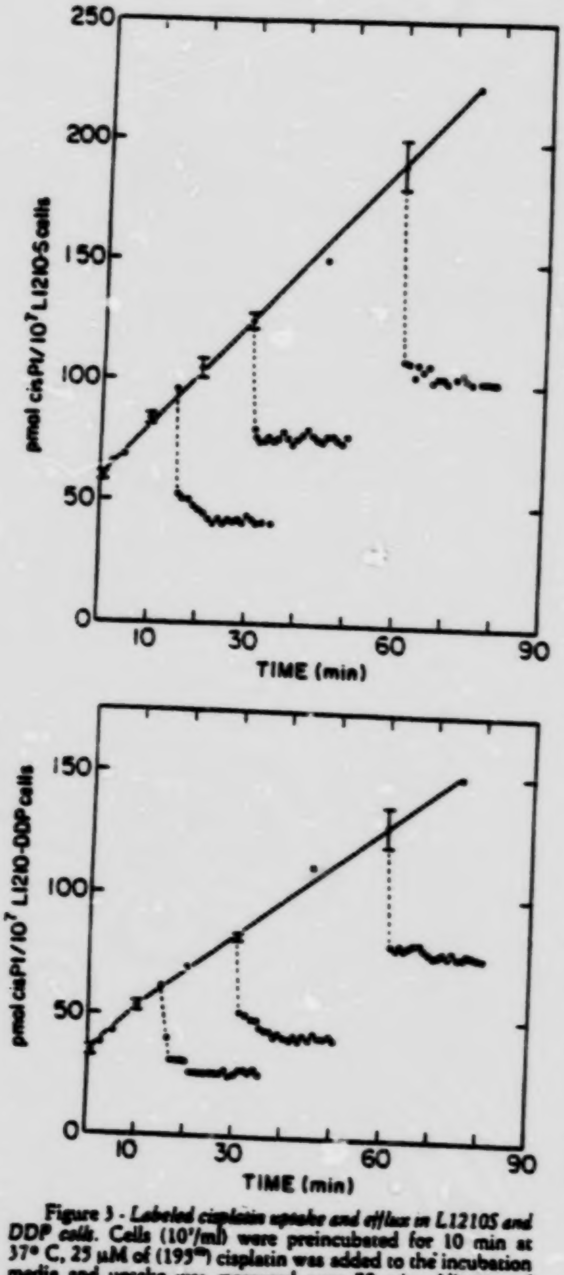

Figure 3 - *Labeled cisplatin uptake and efflux in L1210S and DDP cells.* Cells (10^7/ml) were preincubated for 10 min at 37° C, 25 μM of (195^m) cisplatin was added to the incubation media and uptake was measured over 75 min. Aliquots of (10^6) cells were taken at various time points, washed (3 times) in ice cold saline and counted as described in Methods. Aliquots of the cells were taken at 15, 30 and 60 min. and diluted 20 fold into drug free media. At one min intervals, cells were washed and counted. Efflux measurements were represented by the dotted line. L1210S cells were in Panel A (○) and L1210DDP cells were in Panel B (●):

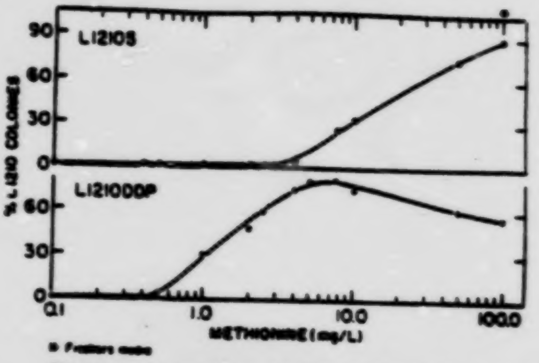

Figure 4 - *Exogenous methionine requirement for optimal L1210S and L1210DDP cell growth.* Cells were seeded at 50 cells/tube for L1210S (○) and L1210DDP (●) in Fischer's media with various concentrations of exogenous methionine (0-100 mg/l). The number of colonies was counted on the 10th day. The cloning efficiency for L1210S and DDP cells under optimal methionine cocnentrations was 90% and 72%, respectively.

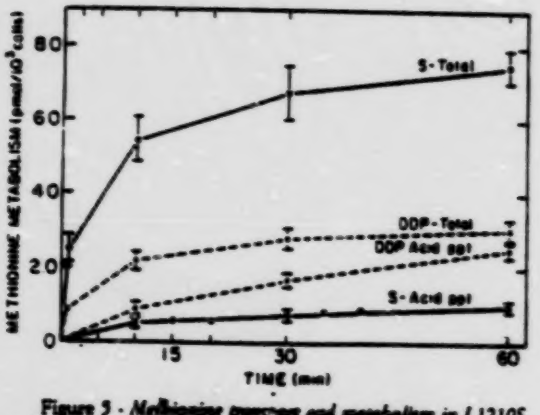

Figure 5 - *Methionine transport and metabolism in L1210S and L1210DDP cells.* L1210S (○) and L1210DDP (●) cells (5×10^6 cells/ml) were incubated with (^{14}C) methionine (500 μM) with 15% dialysed horse serum in Fischer's media depleted of methionine. Aliquots (0-60 min) measured total uptake into the cells and incorporation of labeled methionine into acid insoluble material.

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RESULTS

L1210 cell growth in the presence of cisplatin

The ED_{50} of cisplatin for L1210S was 2.3 µM whereas the ED_{50} for L1210DDP was 23.3 µM (*Figure 1*). The generation time of L1210S cells was 11 (±1) hours and of L1210DDP cells was 14 (±2) hours. Cell volume analysis of L1210DDP by a Coulter Counter channel analyzer revealed a heterogeneous cell population in which the mean cell volume was 25% smaller than the L1210S cells. Yet, there was a similar protein concentration per cell for both cell lines. The resistance to cisplatin was stable since the cells were grown in the absence of the drug for one year and still retained their drug resistant properties. Transplatin, which was less soluble than cisplatin, was tested at similar concentrations and had no significant influence on cell growth for either sensitive or resistant cells (unpublished data).

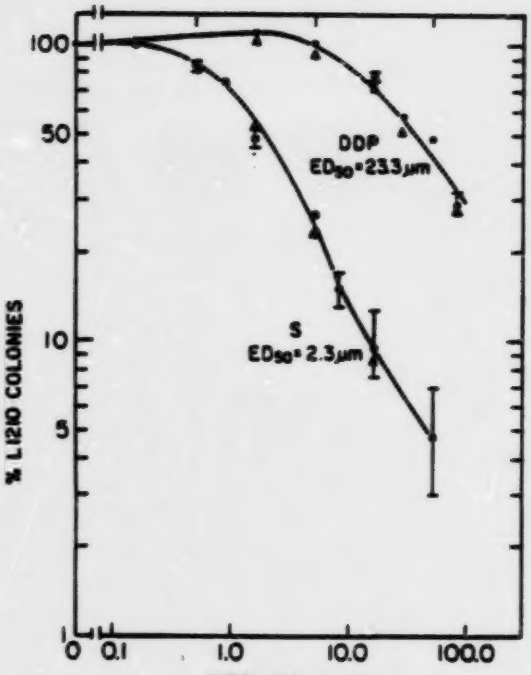

Figure 1 - *Inhibition of L1210 cell growth by cisplatin.* L1210S (○) and L1210DDP (●) cells were grown in various concentrations of cisplatin. After 48 hours of cisplatin exposure the cell number for each concentration was determined. See Methods for details. The ED_{50}'s were calculated as 2.3 µM and 23.3 µM for L1210S and L1210DDP cells, respectively.

Cisplatin uptake in L1210S and DDP cells

The uptake of cisplatin (2 and 10 µM) in both cell lines was similar over the first 10 min (*Figure 2*). The uptake and efflux of labeled cisplatin (25 µM) was measured over 75 min in L1210S and DDP cells (*Figures 3a* and *3b*). The initial binding (background) of cisplatin at 10 sec was significantly (44%) less in the DDP cells. There was an increasing uptake of labeled cisplatin in both cell lines over 75 min which was 24% less in the L1210DDP cells. The rates of efflux of the labeled drug were similar in both. The experiments were done in triplicate and the standard deviation was derived from three separate experiments.

Exogenous methionine requirements for L1210S and L1210DDP cells

Cells grown in Fischer's media and dialyzed serum containing varying concentrations of exogenous methionine had optimal cell growth at 100 mg/l of methionine for L1210S cells while the cocnentration for optimal growth of L1210DDP cells was only 6 mg/l (*Figure 4*). L1210S and L1210DDP cells had cloning efficiencies of 90% and 72%, respectively. Fischer's media contains 100 mg/l of methionine.

Metabolism of methionine in L1210S and L1210DDP cells

^{14}C-methionine (500 µM) uptake and metabolism were measured in Fischer's media in L1210S cells standardized by cell number (*Figure 5*). Methionine uptake in L1210S cells was two- to three-fold greater than in the

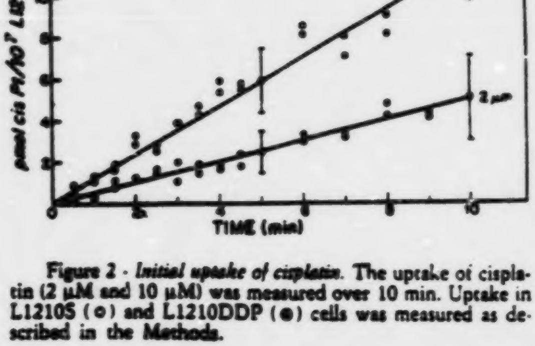

Figure 2 - *Initial uptake of cisplatin.* The uptake of cisplatin (2 µM and 10 µM) was measured over 10 min. Uptake in L1210S (○) and L1210DDP (●) cells was measured as described in the Methods.

excess unlabeled threonine in L1210S. However, there was no significant inhibition in L1210DDP (*Figure 9*). In L1210S cells, threonine was a competitive inhibitor (Ki = 8.3 mM) of sodium dependent MeAIB uptake. Other amino acid competition experiments with methionine and AIB, a substrate for the ASC system, also suggested different substrate specificities between the L1210S and DDP cell lines (unpublished data).

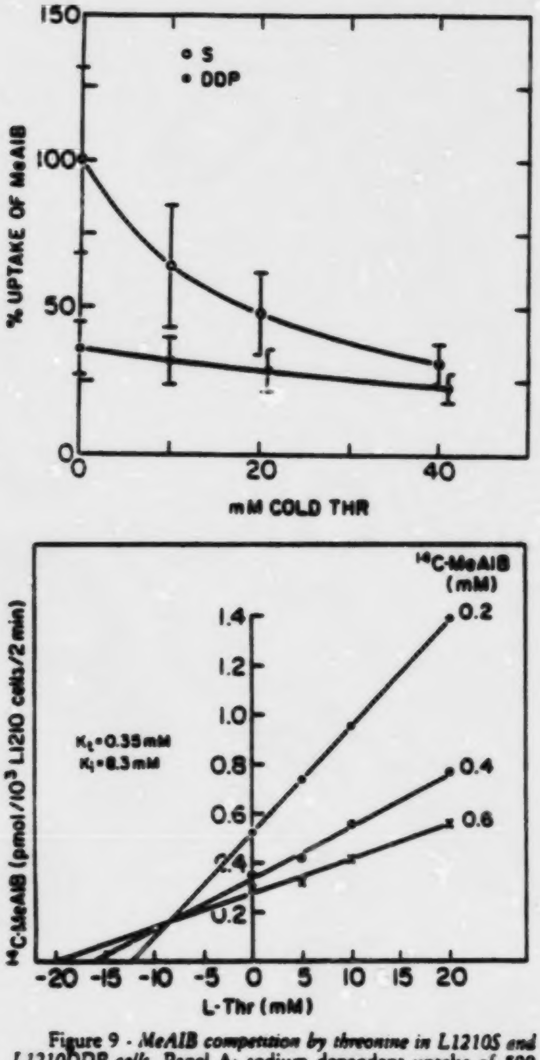

Figure 9 - *MeAIB competition by threonine in L1210S and L1210DDP cells.* Panel A: sodium dependent uptake of 500 µM labeled MeAIB was measured for 3 minutes in the presence of 0, 10, 20 or 40 mM unlabeled threonine in L1210S (o) and L1210DDP (●) cells. The control (100%) was the uptake of MeAIB alone in the L1210S cells. The uptake of MeAIB in L1210DDP cells was a percentage of the L1210S transport. Panel B: sodium dependent uptake of labeled MeAIB (0.2, 0.4, and 0.6 mM) was measured in the presence of unlabeled threonine (5, 10, 20 mM).

DISCUSSION

Since the initial uptake and efflux of cisplatin were found to be similar in both L1210S and L1210DDP cells, impaired drug permeability could not be the mechanism of resistance for cisplatin in L1210 cells. There was a significantly lower initial binding (44%) of the drug to the cell membrane of the resistant cells. This may reflect a decreased number of cisplatin-membrane binding sites in L1210DDP cells. Several cancer chemotherapeutic agents have been shown to cause cytotoxicity by damaging the L1210 cell membrane[4]. Therefore, the role of the membrane in resistance to cisplatin was further investigated.

Both mouse and human tumor cells have a high exogenous methionine requirement for optimal cell growth[4]. Because L1210S cells and a high exogenous methionine requirement[4,12] and L1210DDP cells did not, the membrane transport and metabolism of methionine and several other amino acids were studied. L1210DDP cells had a lower exogenous methionine requirement, a smaller acid-soluble fraction and a faster rate of labeled methionine incorporation into acid insoluble material. Studies of methionine metabolism showed that the high exogenous methionine requirement in L1210S was associated with a large acid-soluble pool of methionine. L1210S cells not only had a high rate of methionine transport, but a large intracellular methionine pool as well. The simultaneous occurrence of a high exogenous requirement and high intracellular pool seems incongruent with basic chemical principles. Yet this imbalance of methionine transport and metabolism has been described as a characteristic of tumor cells[7]. L1210DDP cells had a much lower requirement for exogenous methionine and a much lower methionine transport to metabolism ratio both of which were not characteristic of tumor cells. In L1210DDP cells, decreased methionine transport and increased endogenous methionine biosynthesis correlated with changes in amino acid transport systems. In L1210DDP cells, the uptake of methionine, MeAIB and threonine was decreased in comparison to L1210S cells. The ASC amino acid transport system has been the primary pathway for concentrating methionine and most essential amino acids into mammalian cells [1]. Threonine has been used to define the ASC transport system in tumor cells [3]. In L1210DDP decreased threonine uptake was associated with a change in K_t and with threonine's inability to block MeAIB uptake. In normal rat hepatocytes, threonine and MeAIB were transported by their individual uptake systems, yet in tumor cells threonine competitively inhibited the uptake of MeAIB [3]. In L1210S cells, threonine inhibited MeAIB uptake. Yet L1210DDP cells had separate pathways for threonine and MeAIB transport. This suggested that the membrane transport of resistant cells was more similar to normal cells than leukemic cells in culture.

Although more than one mechanism may explain cisplatin resistance, differences in drug permeability did not appear to be one cause in L1210 cells. In L1210 cells, resistance to cisplatin was associated with changes in both amino acid metabolism and amino acid membrane transport systems.

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Properties of Amino Acid Transport Systems in K562 Cells Sensitive and Resistant to *cis*-Diamminedichloroplatinum(II)[1]

S. Shionoya,[2] Y. Lu, and K. J. Scanlon[3]

Section of Biochemical Pharmacology, Department of Medical Oncology, City of Hope National Medical Center, Duarte, California 91010

ABSTRACT

When K562 cells were made resistant to cisplatin their neutral amino acid transport systems changed. K562 cells cloned in cisplatin developed a 6.7-fold resistance to the drug. The generation times for K562 cells sensitive (S) and resistant (DDP) to cisplatin were similar. The initial uptake and rapid efflux of cisplatin were similar in both cell lines. However, they differed in their sodium dependent neutral amino acid transport properties. In K562S cells the sodium-dependent uptake for methylaminoisobutyric acid was significantly inhibited by threonine (K_i = 10.3 mM), but in K562DDP the uptake of neutral amino acids was significantly lower, and methylaminoisobutyric acid uptake was minimally inhibited by threonine. When K562DDP cells were grown in the absence of cisplatin for 8 weeks, their amino acid transport properties reverted to those of K562S cells. In K562 cells, changes in plasma membrane essential amino acid transport systems were concomitant to both the development of resistance and the redevelopment of sensitivity to cisplatin. Therefore, human malignant cell sensitivity and resistance to cisplatin may be related to membrane nutrient transport systems.

INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II)] has been a potent anticancer chemotherapeutic agent, both as a single agent and in combination regimens (1). Its antitumor activities may be explained in part by the cross-linking reactions involving DNA and protein (2–4) and its effects with cell surface nucleic acids (5) or the plasma membrane (6). Cisplatin has been shown previously to inhibit the uptake of neutral amino acids into the L1210 cells via the ASC-like transport system, while in the L1210 resistant cell line cisplatin was a poor inhibitor of amino acid uptake (6, 7). This permanent alteration of ASC-like transport system in L1210 cells was also associated with a stable resistance to cisplatin (7). In contrast to these mouse cell studies, there are few reports of human cell lines with a stable resistance to only cisplatin (8). Thus, the definitive mechanisms of resistance of human cells to cisplatin remain to be solved. Although the resistance of K562 cells to cisplatin was not stable, data presented in this study suggest that changes in the neutral amino acid transport systems might be correlated to cisplatin cytotoxicity. These interactions with the plasma membrane might account for part of the antitumor activities of cisplatin in human tumors.

MATERIALS AND METHODS

Chemicals. L-[*G*-^{3}H]Threonine (20 Ci/mmol) were purchased from Amersham, Arlington Heights, IL. MeAIB[4] (51.6 mCi/mmol) was purchased from New England Nuclear, Boston, MA. [^{195m}Pt]Cisplatin (145 mCi/mmol) was obtained from Oak Ridge National Laboratories, Oak Ridge, TN (6). Cisplatin (NSF no. 119875-I) was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD.

Cell Culture. K562 cells (K562S) (9) were obtained from Dr. Bertino at Yale University and maintained in Fischer's medium (Grand Island Biological Co., Grand Island, NY) supplemented with penicillin (62.9 mg/l), streptomycin (100 mg/ml), and 10% swine serum (Hyclone, Ogden, UT) (10). Cisplatin-resistant cells (K562DDP) were obtained by cloning in soft agar with 16.5 µM cisplatin and 15% swine serum. The K562DDP cells were maintained either by continuous exposure to 3.3 µM cisplatin in suspension culture or in the absence of drug. Cell growth studies were determined by soft agar cloning of K562 cells in the continuous presence of cisplatin, and colonies were scored at day 12–14 (7).

Uptake and Efflux of Radioactive Cisplatin. The uptake and efflux of [^{195m}Pt]cisplatin into K562S and K562DDP cells were determined with 0.6–1.0 × 10^7 cells/ml in Earle's balanced salt solution as described previously (6). The cell pellet was dissolved in 6.5 ml of Scinti-Verse (Fisher Scientific, Fair Lawn, NJ) and counted by Liquid Scintillation System LS3801 (Beckman Instruments, Inc., Somerset, NJ).

Amino Acid Transport Studies. The net sodium-dependent amino acid transport was determined by subtracting the sodium-independent transport from the total transport in the presence of sodium (6). Incubation medium for determining sodium-independent transport was Earle's balanced salt solution in which 116 mM choline chloride was substituted for 116 mM NaCl. Uptake of labeled amino acids (threonine and MeAIB) was determined by measuring the incorporation of radioactivity into the cell pellet. Log-phase cells were resuspended in the incubation medium to a density of 2.5 × 10^6 cells/ml, and 200-µl aliquots were withdrawn at timed intervals after adding labeled amino acids (6). The cell pellets were separated immediately by centrifugation through a gradient of an oil (2 parts silicone: 1 part dinonylphthalate) (11) into 12% perchloric acid, dissolved in 6.5 ml of Scinti-Verse, and counted by Liquid Scintillation System LS3801. The initial velocity was determined by linear-least squares fit of the uptake *versus* time. The kinetic parameters (K_t and V_{max}) were determined by an iterative non-linear least-squares program to the direct Michaelis-Menten equation. The data were not weighed (12). K_t has been defined as the concentration of the amino acid substrate required for one-half saturation of a mediated-transport system (13).

RESULTS

Tumor Cell Growth in the Presence of Cisplatin. Cisplatin inhibited the colony formation of K562S and K562DDP cells with an ED_{50} (concentration which induces 50% inhibition of cell proliferation) of 0.67 and 4.5 µM, respectively (Fig. 1). The K562DDP cells were 6.7-fold resistant to cisplatin, and the resistance was unstable. Within 4 weeks of being grown in drug free medium, the K562DDP cells were again equally as sensitive to cisplatin as were the parent cells (Fig. 2). The generation time for K562S and K562DDP cells in culture was 22.2 ± 0.8 (SD) and 25.4 ± 2.3 h, respectively.

Initial Uptake and Efflux of [^{195m}Pt]Cisplatin. The uptake of cisplatin (1 and 5 µM) during the first 7.5 min was similar in both K562S and K562DDP cells (Fig. 3). The uptake of cisplatin (25 µM) into K562S and K562DDP cells increased with time over 75 min (Figs. 4 and 5). In the resistant cells, the initial binding of cisplatin to the cell was 13% less when compared to the sensitive cells (Figs. 4 and 5). The net uptake

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[2] Present address: Saitama Medical Center, Kawagoe-shi, Saitama 350, Japan.

[3] Scholar of the Leukemia Society of America. To whom requests for reprints should be addressed.

[4] The abbreviations used is: MeAIB, α-[1-^{14}C]methylaminoisobutyric acid.

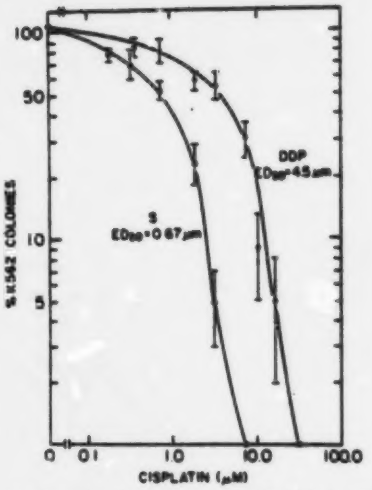

Fig. 1. Colony formation in the presence of cisplatin. K562S (○) and K562DDP (●) cells were grown in the presence of six concentrations of cisplatin, and the number of K562 colonies was determined at 12–14 days in soft agar. See "Materials and Methods" for details. *Bars*, SE.

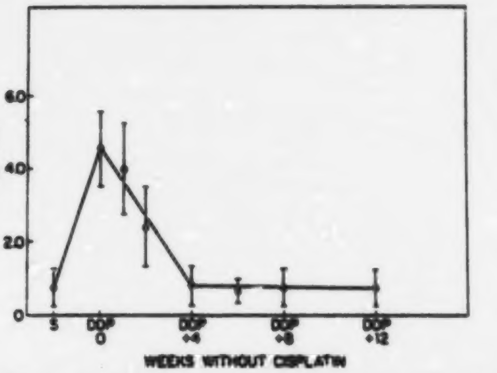

Fig. 2. Cisplatin resistance in K562 cells. K562 cells were grown in the presence of six concentrations of cisplatin in soft agar, and colonies were counted at 12–14 days. This measurement was made in K562S and K562DDP cells when grown in the absence of cisplatin for 1 to 12 weeks. These data were obtained from three separate experiments in duplicate. *Bars*, SE.

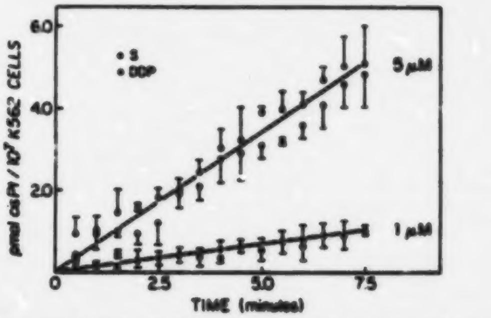

Fig. 3. The initial uptake of cisplatin (1 and 5 μM) was measured over 7.5 min. Uptake in K562S (○) and K562DDP (●) cells was measured as described in "Materials and Methods." *Bars*, SE.

of cisplatin at 75 min was 20% less in the resistant cell line than in the sensitive cells.

Cisplatin uptake in the K562 cells was temperature-sensitive (Figs. 4 and 5). There was a 60% decrease in cisplatin uptake at 24°C when compared to 37°C at 40 min.

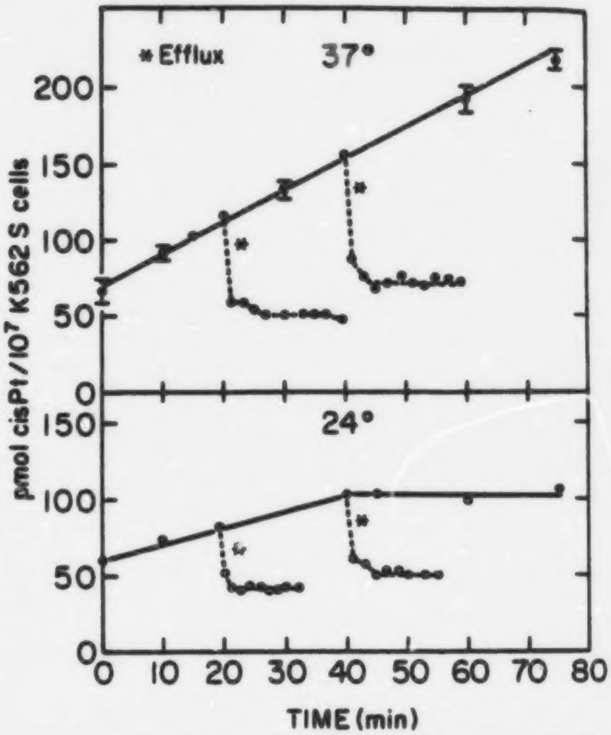

Fig. 4. The uptake and efflux of cisplatin from K562S cells. The K562S (○) cells were incubated at 37°C or 24°C with 25 μM cisplatin. The uptake was measured over 75 min. The efflux (----) of cisplatin (○) was measured at 20 and 40 min by diluting the K562 cells 20-fold with drug free medium at either 37°C or 24°C. *Bars*, SE.

The efflux of cisplatin at 37°C and 24°C was rapid and complete in 2–5 min (Figs. 4 and 5). The efflux was measured by diluting cells 20-fold into drug free medium and measuring the amount of radioactivity associated with the cells over 20 min. With increasing time, there was an increased association of cisplatin with the K562 cells that could not be effluxed from the cells. The rate of cellular retention for radioactively labeled cisplatin between sensitive and cisplatin resistant cells appeared to be similar. There was significantly less uptake and cellular retention of cisplatin at 24°C; similar results were obtained for the K562DDP cells.

Sodium-dependent Amino Acid Transport. The sodium-dependent uptake of the amino acids was significantly lower in K562DDP cells than in K562S cells. MeAIB uptake was exclusively sodium-dependent. V_{max} and K_t values in S-cells were 0.42 ± 0.02 pmol/10^3 cells/30 s and 355.8 ± 34.6 μM, and those in DDP cells were 0.17 ± 0.005 pmol/10^3 cells/30 s and 150.3 ± 11.8 μM (Fig. 6). K_t values for threonine were 171 ± 16 μM in S and 332 ± 49 μM in DDP for sodium-dependent uptake (Fig. 7).

Inhibition of MeAIB Uptake by Threonine. Threonine was a potent inhibitor in MeAIB uptake in K562S cells with a K_i of 10.3 ± 0.5 mM (Fig. 8), whereas in K562DDP cells its inhibitory effect was significantly reduced (Fig. 9). When K562DDP cells were maintained in the drug-free medium for 8 weeks, MeAIB uptake reverted to about 70% of that of S-cells, and threonine again strongly inhibited MeAIB uptake (Fig. 9). Membrane transport changes could be seen as early as 1 week, and by 10 weeks sodium dependent transport of MeAIB closely resembled the parent K562S cells. When K562S cells were incubated with

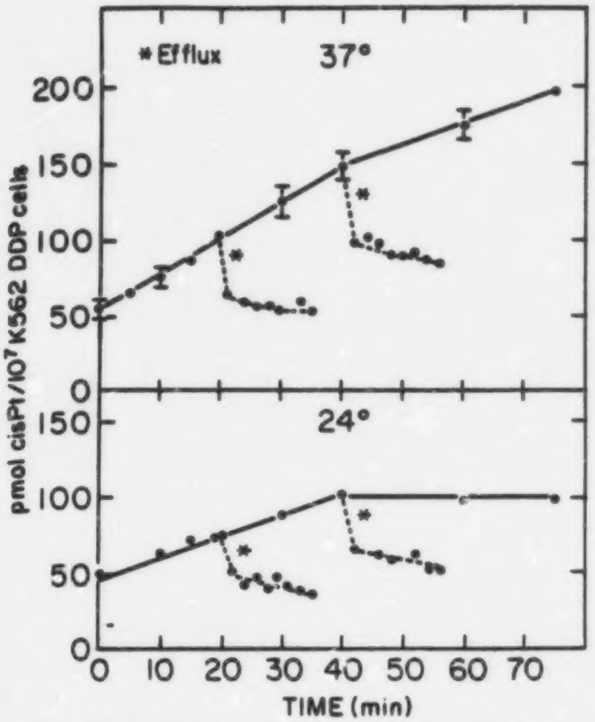

Fig. 5. The uptake and the efflux of cisplatin from K562DDP cells. The K562DDP (●) cells were incubated at 37°C or 24°C separately with 25 μM cisplatin. The uptake was measured over 75 min. The efflux (----) of cisplatin (●) was measured at 20 and 40 min by diluting the K562 cells 20-fold with drug free medium at either 37°C or 24°C. *Bars*, SE.

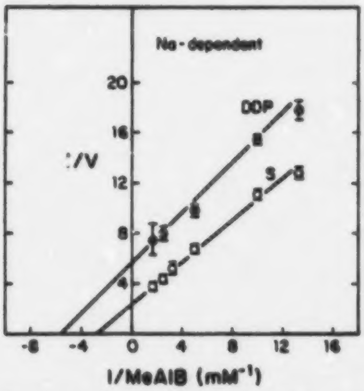

Fig. 6. Initial MeAIB uptake in K562S and K562DDP cells. Sodium dependent initial uptake (1 min) of MeAIB at various concentrations (75–600 μM) was measured in K562S (O) and K562DDP (●) cells. V was expressed as pmol/10⁵ cells/0.5 min. *Bars*, SE.

cisplatin (10 μM) for 15 min at 37°C there was a decrease in the sodium dependent uptake of threonine, MeAIB, and methionine by 57.6, 33.1, and 28.3%, respectively (Table 1). Neutral amino acid uptake in K562DDP cells was not significantly influenced by cisplatin (Table 1).

DISCUSSION

In this study, cisplatin cytotoxicity in K562 cells did not correlate with drug permeability but with the expression of the essential sodium dependent amino acid transport systems.

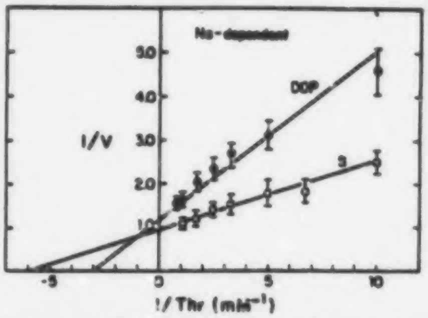

Fig. 7. Threonine uptake in K562S and K562DDP cells. Sodium dependent uptake of threonine at various concentrations (100–1200 μM) was measured in K562S (O) and K562DDP (●) cells. V was expressed as pmol/10⁵ cells/0.5 min. *Bars*, SE.

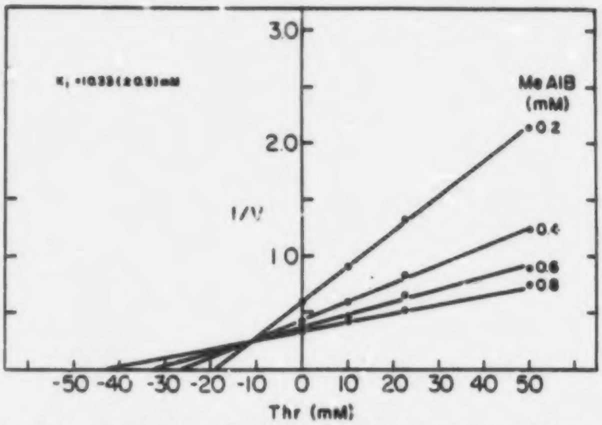

Fig. 8. MeAIB uptake in the presence of unlabeled threonine in K562S cells. Sodium dependent uptake of labeled MeAIB (0.2, 0.4, 0.6, and 0.8 mM) was measured in the presence of unlabeled threonine (0, 10, 25, and 50 mM).

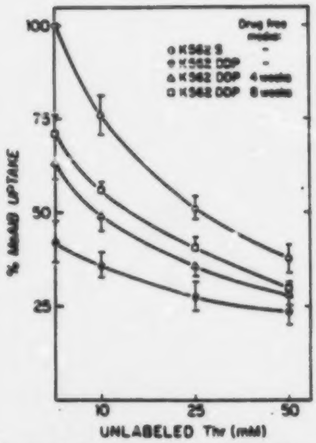

Fig. 9. MeAIB competition by threonine in K562S and K562DDP cells. Sodium dependent uptake of 500 μM labeled MeAIB was measured for 4 min in the presence of 0, 10.25, or 50 mM unlabeled threonine in K562S or K562DDP cells. The control (100%) was the uptake of MeAIB alone in the K562S cells. *Bars*, SE.

K562DDP cells were established by soft agar cloning in the presence of cisplatin. However, these cells could revert to the drug sensitive parental phenotype within 4–8 weeks if they were maintained in the drug-free medium. This finding contrasts with L1210 murine leukemia cells, which had a stable resistance

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Table 1 *Influence of cisplatin on amino acid uptake in K562 cells*

K562S and K562DDP cells were preincubated for 15 min with cisplatin (10 μM) at 37°C. The initial uptake for methionine in K562S and K562DDP was 2.8 and 1.2 pmol/10^7 cells/min, respectively. The initial uptake for MAIB and threonine was obtained from Figs. 6 and 7, respectively. The sodium dependent uptake was measured as described previously (6, 7).

Amino acid (500 μM)	Cisplatin (μM[a])	% inhibition of sodium dependent uptake	
		K562S	K562DDP
Threonine	10	56.7 ± 14[b]	<5
MeAIB	10	33.1 ± 8	<5
Methionine	10	28.3 ± 10	<5

[a] Pretreatment for 15 min at 37°C.
[b] Mean ± SE.

to cisplatin and a permanently changed ASC amino acid transport system (7). Uptake of cisplatin was temperature sensitive, with the maximum uptake at 37°C. The initial uptake and efflux of cisplatin were similar between sensitive and resistant K562 cells (Fig. 3), as also obtained for murine leukemia cells (7). These data would suggest that cisplatin permeability does not play a significant role in the initial step of resistance in K562 cells. Neither verapamil nor DL-butionine-*S,R*-sulfoximine play a role in reversing cisplatin resistance in K562 cells (data not shown).

Exogenous methionine is an absolute requirement for K562 cell growth (14), and cisplatin inhibits methionine uptake both in L1210 cells and in K562 cells. If the membrane transport system is damaged, then the cell must endogenously synthesize more methionine temporarily for growth. The potential elevation of the methylenetetrahydrofolate pool for methionine biosynthesis may play a role in the synergism noted clinically between cisplatin and fluorouracil (15). This is currently under investigation.

In L1210 cells, cisplatin has caused changes in both methionine transport and metabolism (6, 7). Methionine is primarily transported into tumor cells by the ASC amino acid transport system (16). The specificity of ASC amino acid transport systems has been characterized by threonine inhibition (K_i = 16 mM) of MeAIB uptake in rat HTC tumor cells (14). However, the corresponding normal rat hepatocytes lacked this overlapping transport system for MeAIB and threonine. Threonine was also a potent inhibitor (K_i = 8.3 mM) of MeAIB uptake in L1210 cells, while the cisplatin resistant cells lacked this property (6, 7). In K562S cells, threonine was an inhibitor (K_i = 10.3 mM) of MeAIB uptake, while the resistant cells lacked the ability of threonine to inhibit MeAIB uptake. These data would suggest that some types of tumor cells have different substrate specificity for the ASC amino acid transport system when compared to normal cells or drug resistant cells (6, 7). This could be the basis for new drug design or a new treatment strategy. Cisplatin's damage to the cell membrane and the amino acid transport systems was transient and could be repaired in 4–8 weeks, as noted by the cells reverting to the parental K562S phenotype. It would be an oversimplification to suggest that cisplatin cytotoxicity and resistance is due solely to a single membrane effect. Cisplatin damage to the DNA and its subsequent repair of DNA may also account for this phenomenon, since it has been well documented that cisplatin binds to DNA (2–5).

In summary, there appeared to be a temporal relationship between the expression of the sodium-dependent amino acid transport systems and cisplatin cytotoxicity in sensitive and resistant K562 cells.

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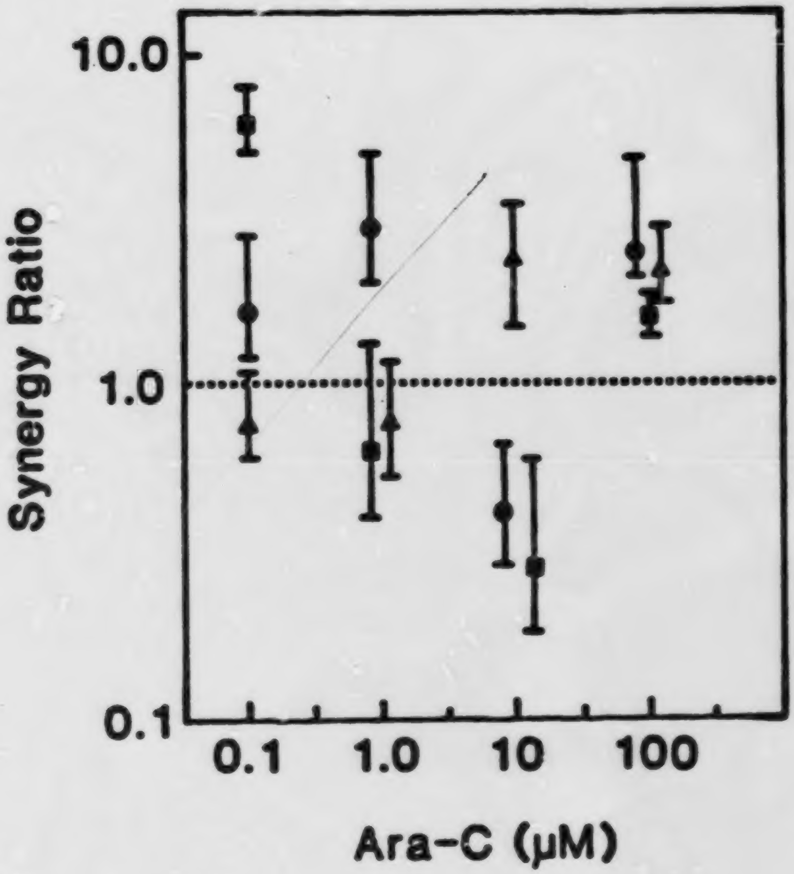
High doses of 1-β-D-arabinofuranosylcytosine, administered as a continuous intravenous infusion, were evaluated in a Phase I trial in 14 patients with advanced solid tumors. 1-β-D-arabinofuranoslycytosine was given at 250 mg/M^2/hour for infusions of 12 to 36 hours. The mean steady state ara-C plasma level was 19.6 μM with a range of 9 μM-59 μM. The principal toxicity was myelosuppression. An infusion of 18 hours was well tolerated by most patients. A Phase II dose of 250 mg/M^2/hr for 24 hrs. can be employed if platelet support is available. This dose schedule may be useful in the treatment of hematologic disorders or in clinical combinations with DNA damaging agents in the treatment of solid tumors.

1-B-D-arabinofuranosylcytosine (ara-C) incorporates into replicating cellular DNA and the extent of this incorporation correlates with loss of clonogenic survival. More recent findings have demonstrated that incorporation of ara-C into DNA undergoing repair of damage induced by UV light also results in cell lethality. On the basis of previous studies demonstrating a marked synergism between CDDP and ara-C in LoVo colon carcinoma cells, the present work has examined the interaction of these agents at a biochemical and cellular level in the MCF-7 human breast carcinoma line. The extent of ara-C incorporation into MCF-7 DNA correlates significantly with loss of clonogenic survival in a dose dependent manner. The effects of CDDP on the formation of MCF-7 (ara-C)DNA were monitored using both cesium sulfate and cesium chloride density centrifugation. The results demonstrate that CDDP had little, if any, detectable effect on incorporation of ara-C into DNA. Furthermore, combinations of CDDP and low concentrations of ara-C (10^{-7} and 10^{-6}M) decreased MCF-7 clonogenic survival in an additive but not synergistic manner. Modest synergy was detectable with CDDP and higher ara-C concentrations (10^{-5} and 10^{-4}M). The interaction between CDDP and ara-C ~~is~~ was apparently dependent on concentration, duration of exposure and cell type. There ~~is~~ was not dramatic synergy between CDDP and ara-C in MCF-7 cells. These findings may be relevant to the design and interpretation of CDDP/ara-C clinical trials.



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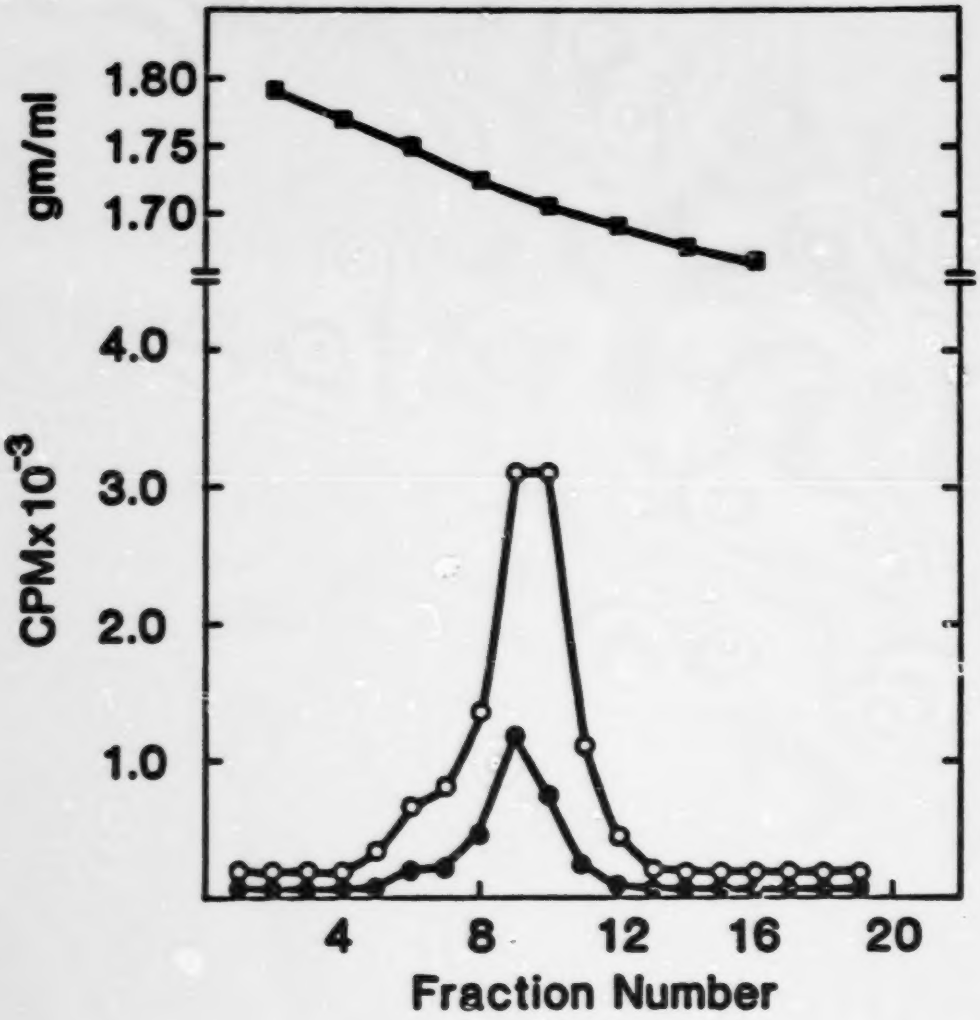

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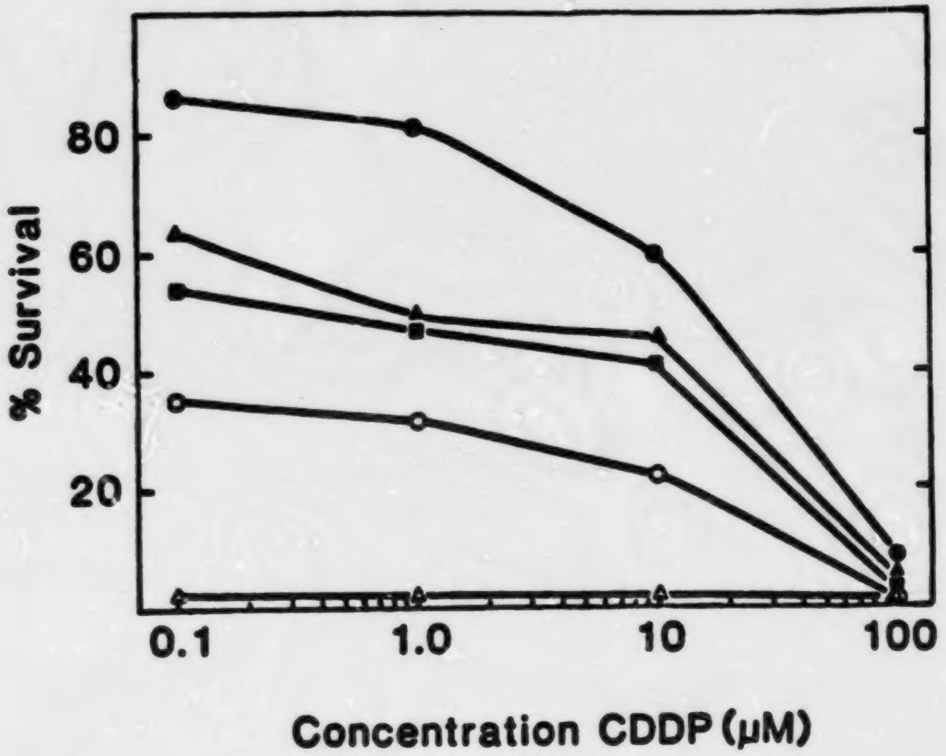

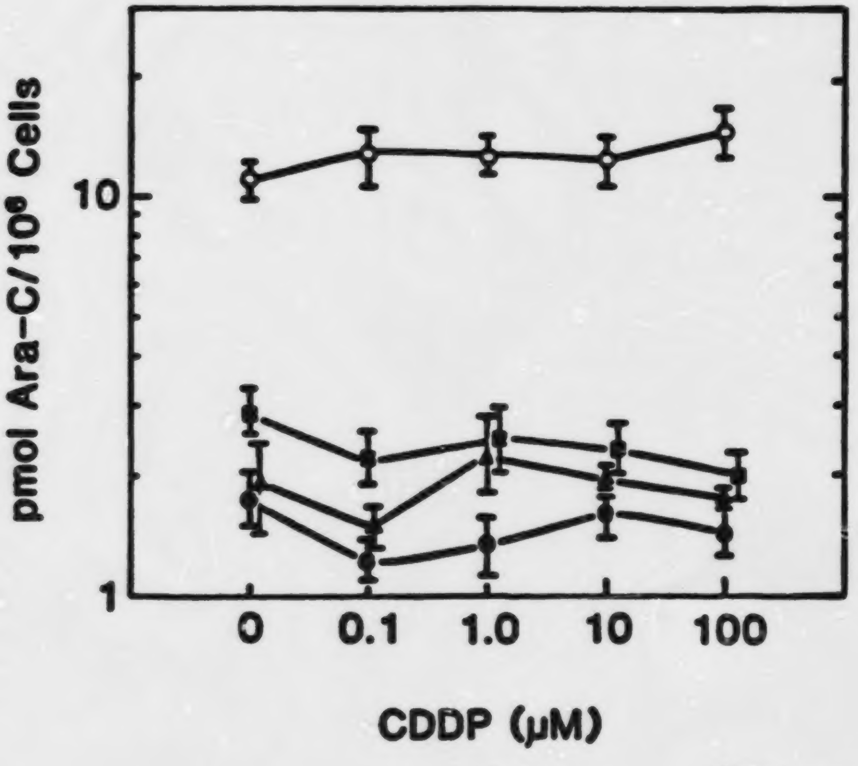

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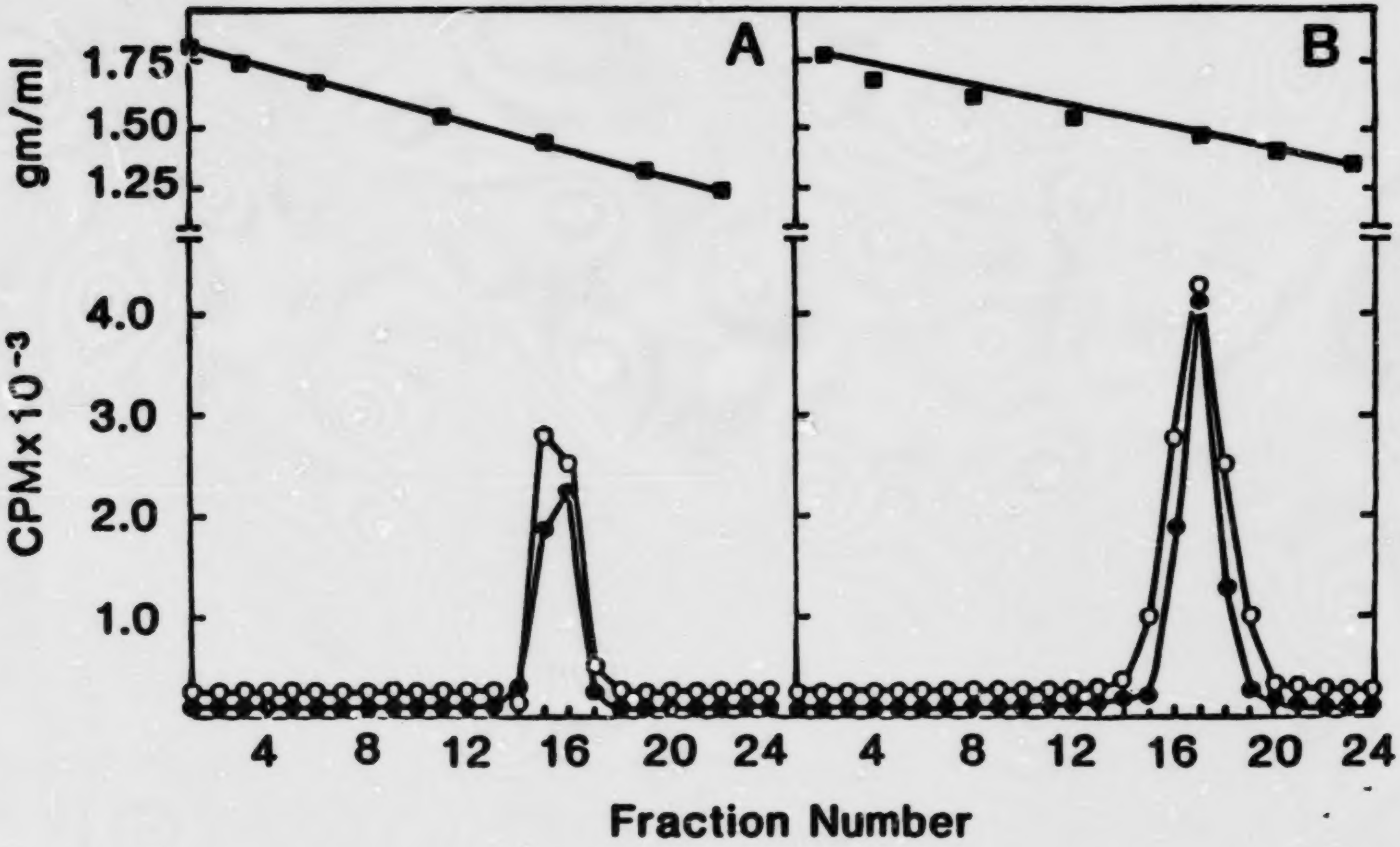

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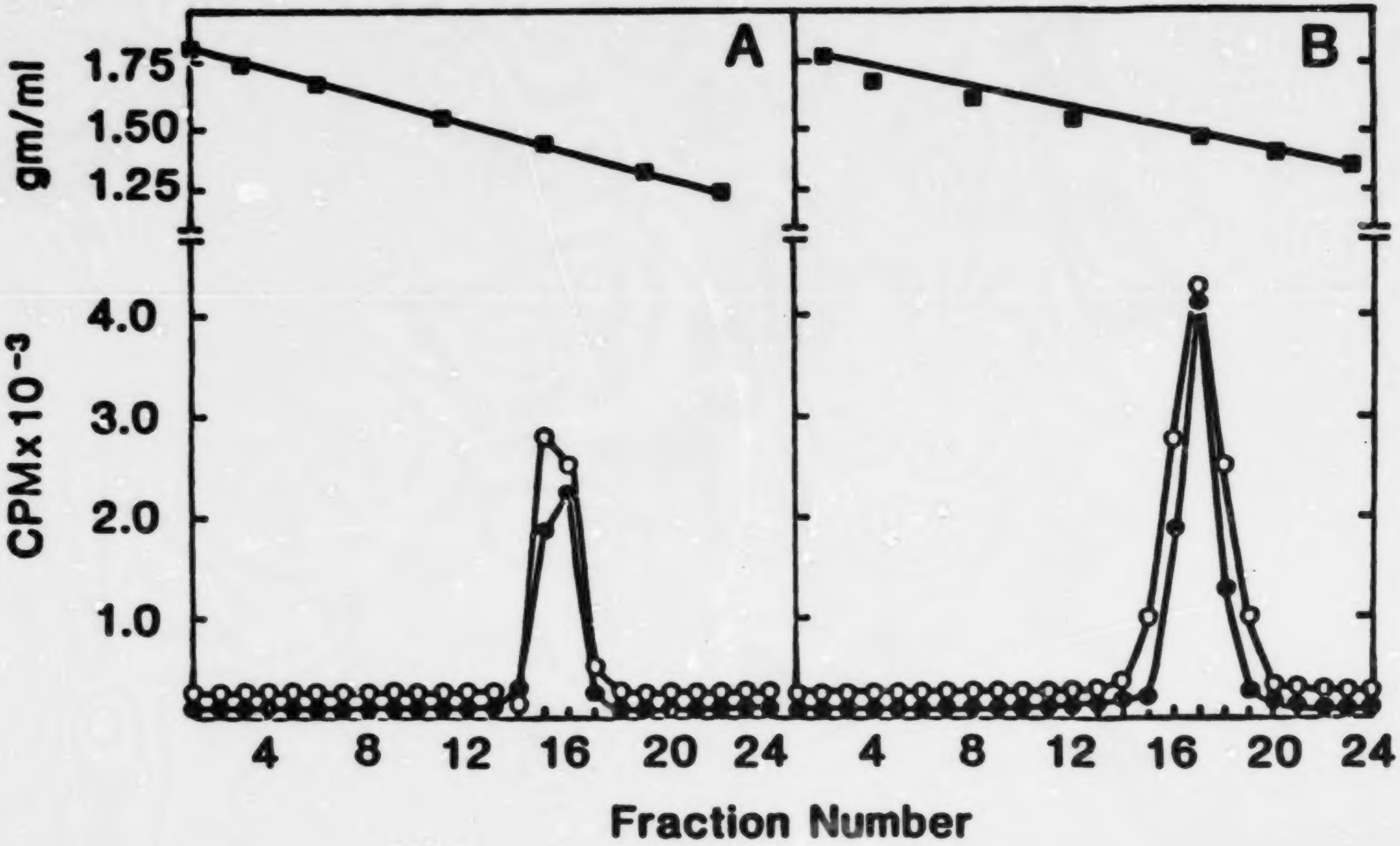
FIGURE 4


A
B
gm/ml
1.75
1.50
1.25
CPMx10^{-3}
4.0
3.0
2.0
1.0
4
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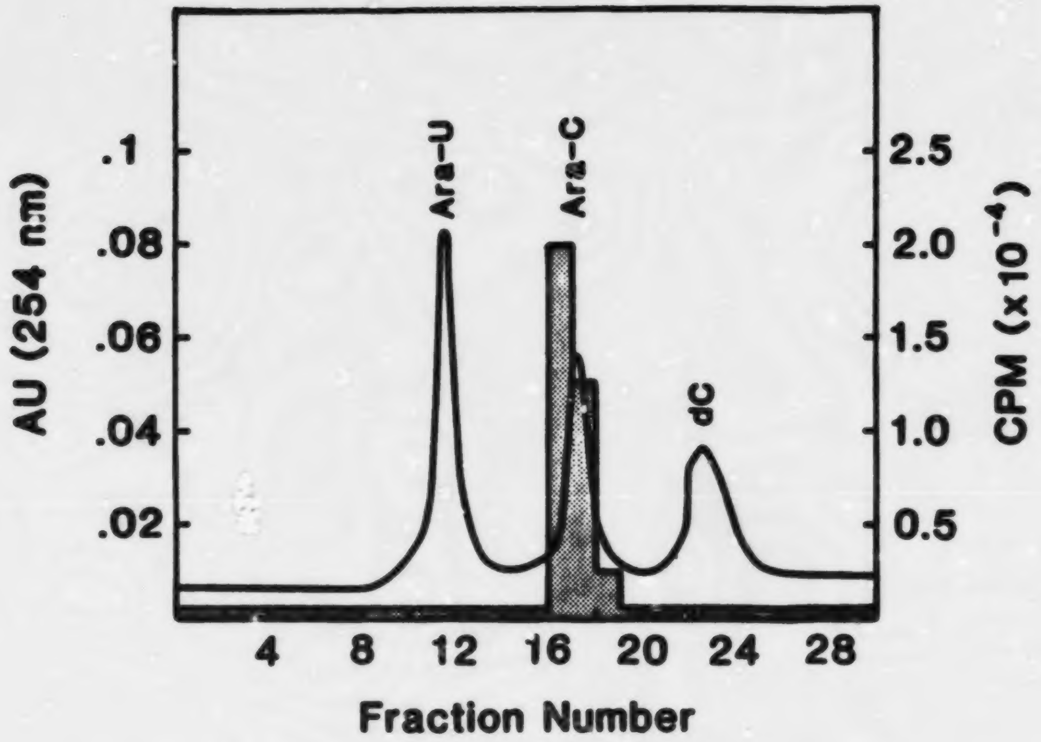
Ara-U
Ara-C
dC
AU (254 nm)
.1
.08
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CPM (x10^{-4})
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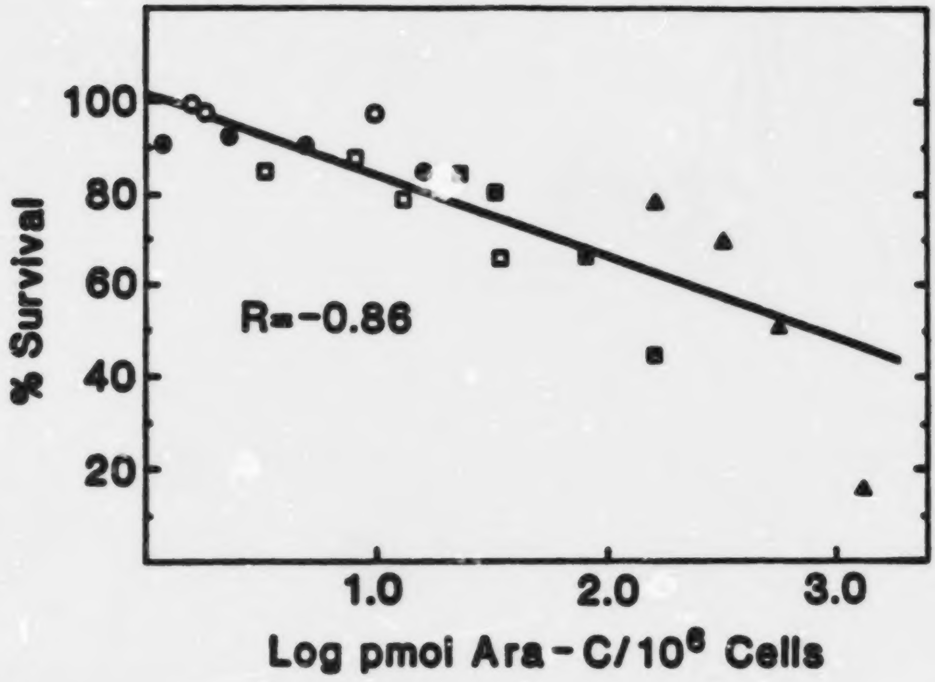
% Survival
100
80
60
40
20
R=-0.86
1.0
2.0
3.0
Log pmol Ara-C/10^6 Cells

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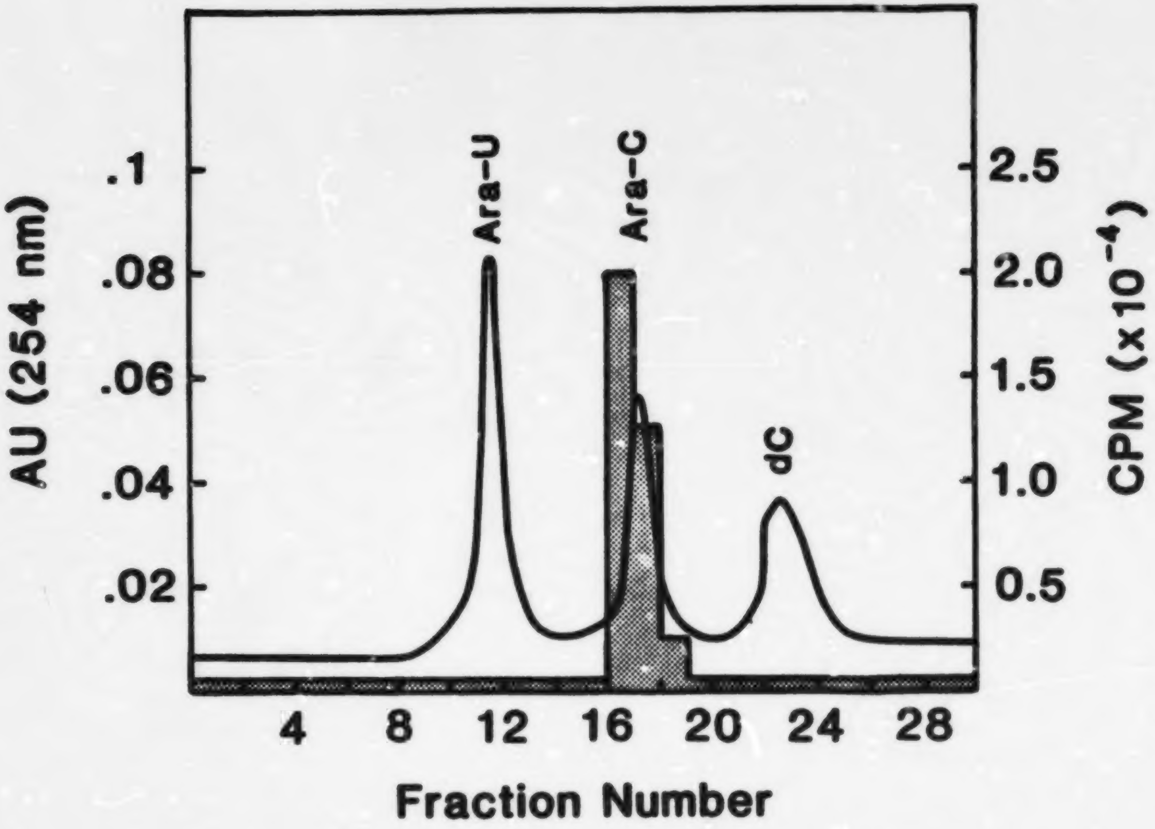

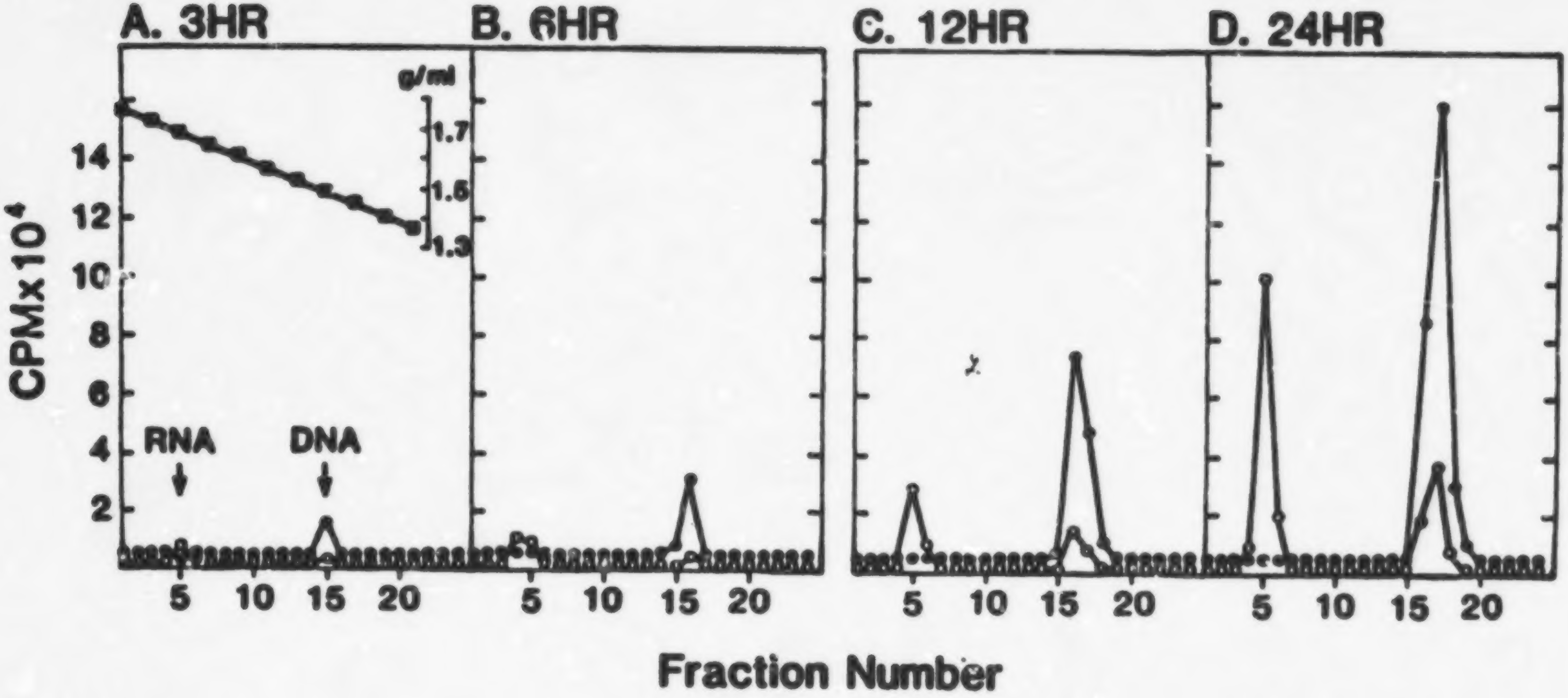

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TABLE 1

PATIENT CHARACTERISTICS

No. of Patients	14
No. of Courses	28
Median Age (Range)	52 (23-69)
Median No. Prior Therapies	2
Performance Status	
0-1	9
2-3	5
Primary Sites	
Breast	4
Colon	4
Lymphoma	4
Lung	1
Sarcoma	1

TABLE 2

MEAN PLASMA LEVELS FOR ARA-C AND ARA-U

PATIENT	DURATION	PLASMA ARA-C ± SEM (U/MOL/L)	PLASMA ARA-U (uMOL/L)
A	12	59.5 ± 2.78	186
B	12	9.0 ± 1.5	176
	12	8.1 ± 0.23	159
C	12	27.3 ± 2.00	248
D	12	21.3 ± 0.85	299
	12	11.8 ± 1.32	278
E	18	23.3 ± 2.10	183
F	18	22.7 ± 2.45	-
G	18	11.2 ± 0.73	183
	24	34.1 ± 4.15	139
H	18	22.7 ± 4.45	177
	24	15.8 ± 3.10	156
I	24	13.1 ± 0.84	190
J	24	25.8 ± 1.51	243
K	24	13.1 ± 1.97	211

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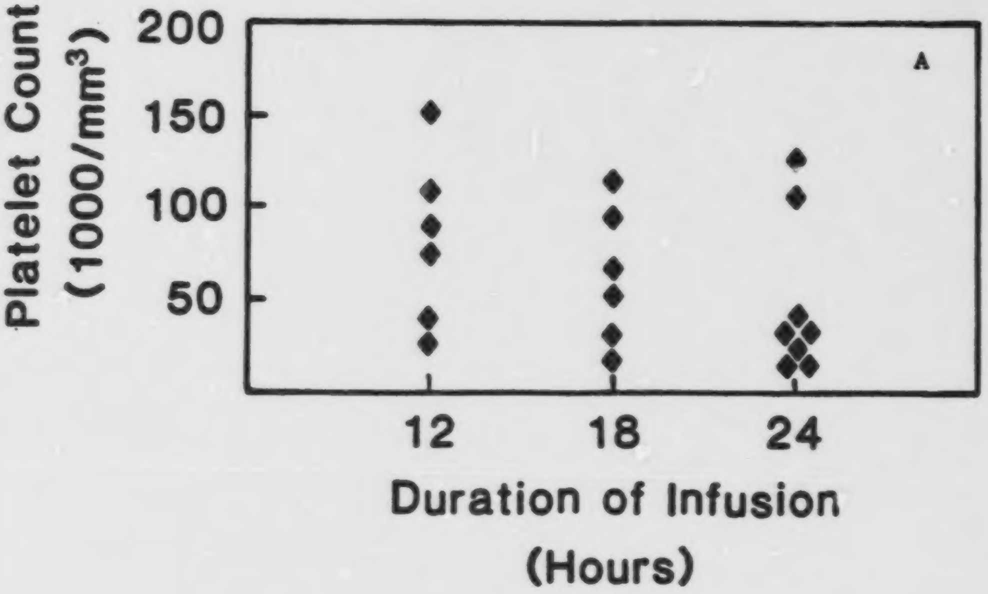

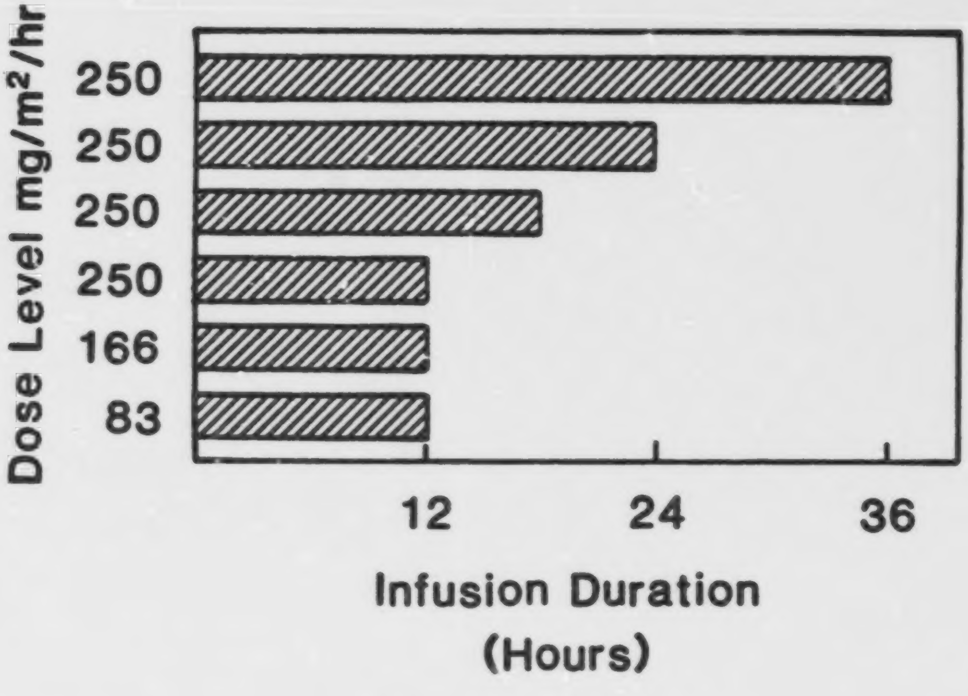

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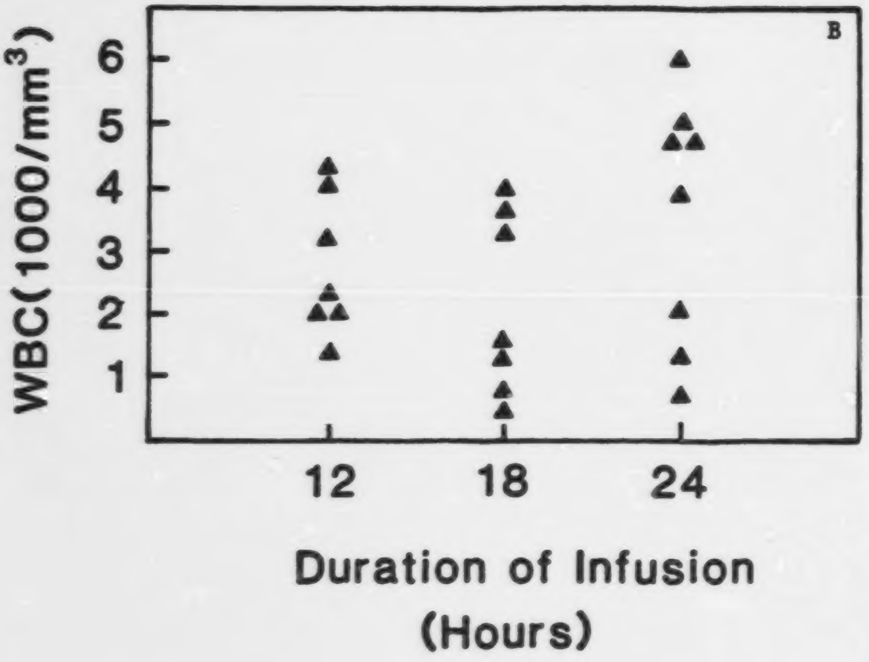

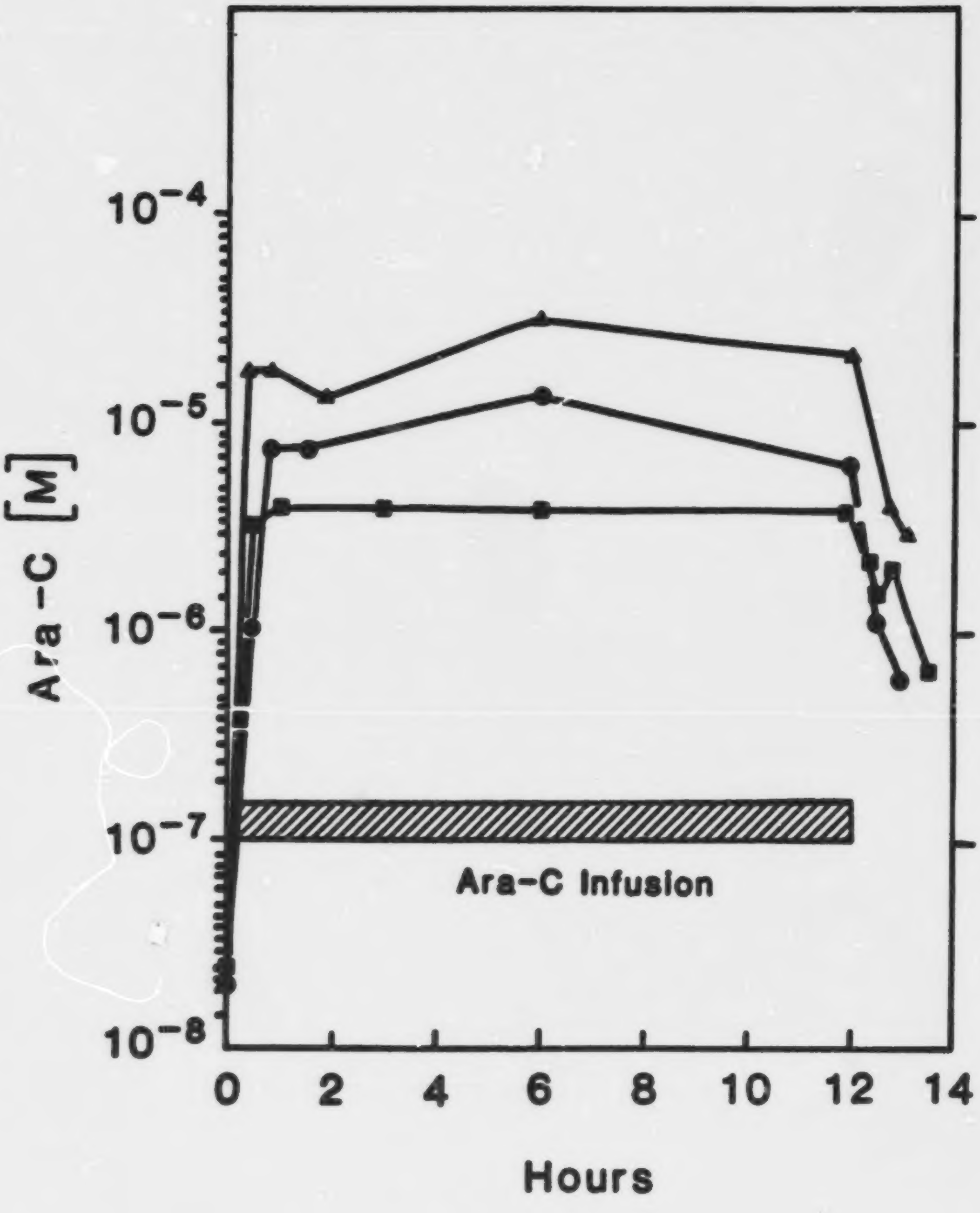

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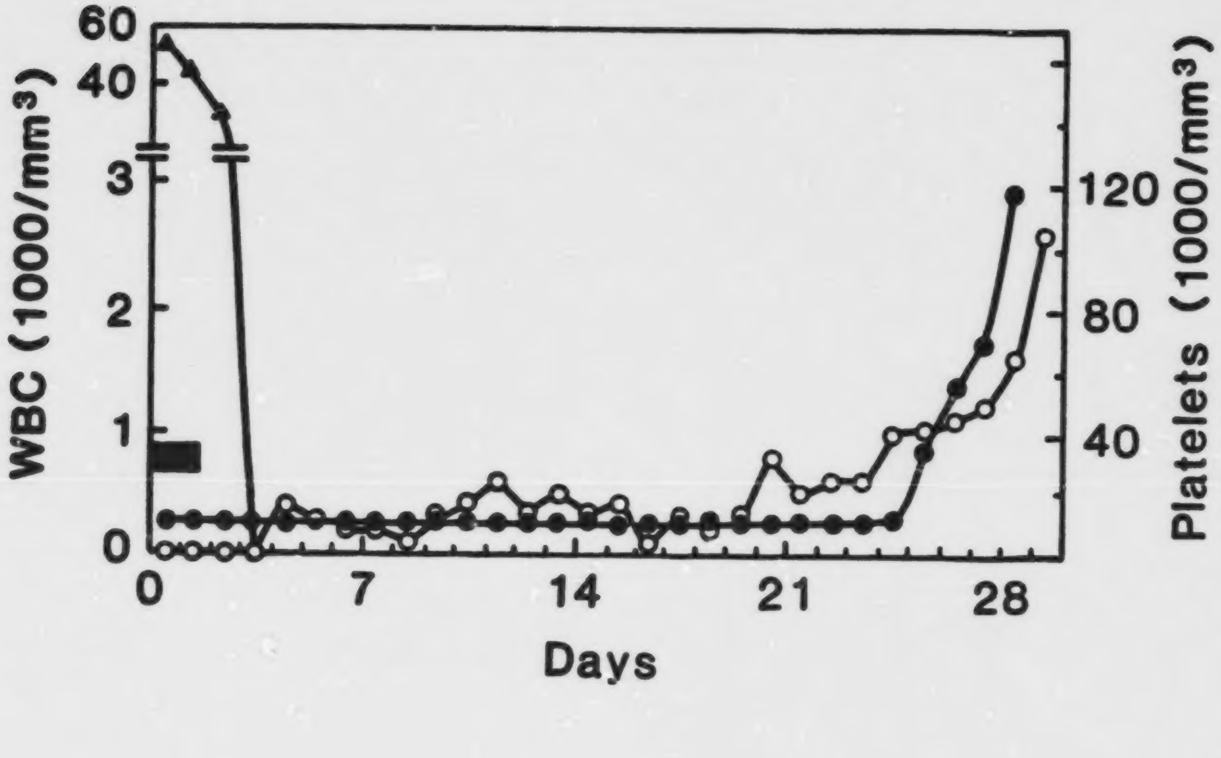
60
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WBC (1000/mm³)
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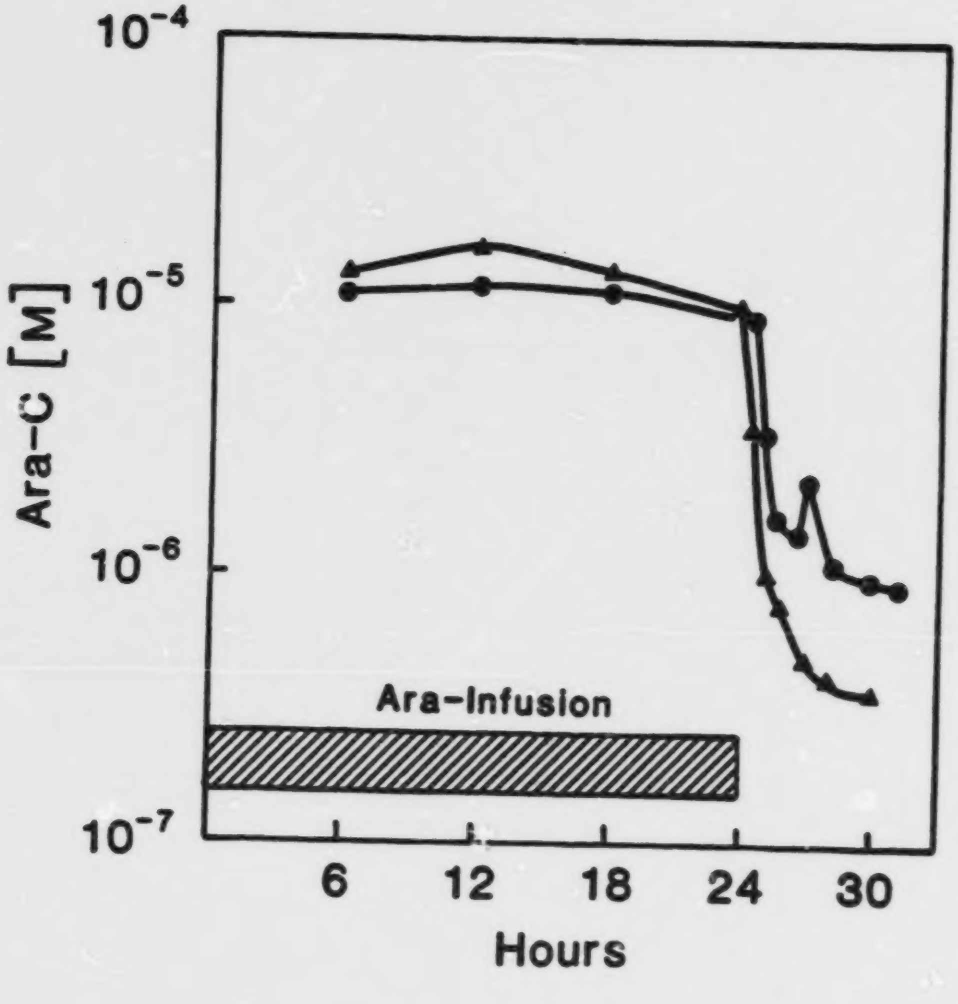

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M.D. Anderson
July 1986

PLATINUM AND NUCLEOSIDES: STUDIES WITH ARA-C

Previous studies conducted in our laboratory documented a marked synergistic lethal effect for the combination of cis-diamminedichloroplatinum (cis-DDP) and cytosine arabinoside (ara-C) with a killing enhancement of almost 63-fold over that predicted from the additive effect of each drug alone [1,2]. The combination had no greater effect on inhibition of DNA synthesis, ara-C metabolic pathways, or ara-C incorporation into nucleic acids than the additive effect of each agent alone [3]. Neither caffeine or ara-C alone, or the paired combination of these two agents was lethal and caffeine failed to produce a significant increase in the cell killing efficacy of cis-DDP. In contrast, the triple combination of cis-DDP, caffeine and ara-C was significantly more lethal than the predicted additive effect of each of the three agents alone and almost 5-fold more effective than the pair ara-C: cis-DDP. These findings suggested that: (i) the primary damage induced by cis-DDP is minimally, if at all, repairable by a caffeine-sensitive mechanism [4]; (ii) the injury caused by the ara-C:cis-DDP combination is possibly of a different physiocochemical nature than that induced by cis-DDP alone; (iii) only the latter type of injury is amenable to repair by caffeine-inhibited repair mechanisms [5].

The nature of the damage induced by the combination of cis-DDP and ara-C was further analyzed by the alkaline elution technique of Kohn et al. [6]. Established human colon cancer cells (LoVo) treated with cis-DDP alone showed a considerable degree of DNA-crosslinks (DNA-CL) while cells treated simultaneously with cis-DDP and ara-C showed an even greater CL effect [3]. The DNA-CL produced by the combination formed earlier [7] and were more resistant to DNA repair [2] than those induced by cis-DDP alone. In contrast,

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the degree of CL induced by the trans-isomer of platinum, either alone or in combination with ara-C was only marginal, demonstrating that the interaction between ara-C and platinum complex required the specific cis structure.

No significant lethal effects were elicited by any natural nucleoside (thymidine, TdR; uridine, UdR; cytidine, CdR; and adenosine, AdR) administered alone when exposed to the cells for 1 h at 37°C in concentrations ranging up to 1000 μg/ml. Under the same treatment conditions, cis-DDP killed about 70% of the cells at a concentration of 5 μg/ml, and about 90% at a concentration of 10 μg/ml. trans-DDP killed only 10% of the cells at a concentration of 10 μg/ml and about 80% at 25 μg/ml. There were no significant increases in cytotoxicity for cells treated with any combinations of trans-DDP (either 10 or 25 μg/ml) and nucleosides. Alkaline elution experiments showed no DNA retention of cells treated with any of the nucleoside alone and only minimal (CL factor = 1.4) for trans-DDP alone. In contrast, the combination of cis-DDP with each nucleoside was eminently synergistic. At concentrations of 5 μg/ml of cis-DDP and 1000 μg/ml of nucleoside, the increase in killing effect with respect to the sum of each agent administered alone was 10-fold greater for TdR and AdR, and 5-fold greater for both UdR and CdR.

Changes in alkaline elution patterns over time following treatment with either cis-DDP, or combinations of cis-DDP with nucleosides, were similar to those described before [2,3], that is, rising CL to peak values at about 6 h and decreasing subsequently to a minimum at 24 h after treatment. However, CL induced by the combinations were always greater than those resulting from treatment with cis-DDP alone, even when measured as early as 3 h after treatment. In contrast, there was no major differences in the rising or descending CL factor slopes for any of the cis-DDP and nucleoside combinations or descending CL factor slopes for any of the cis-DDP and nucleoside combinations indicating similar rates of CL formation and repair. Therefore, for all subsequent experimental results only the CL factor obtained 6 h after treatment was considered. DNA elution decreased as a function of increasing concentrations of TdR in combination with cis-DDP, suggesting increments in DNA-CL. Similar results were obtained for every combination of cis-DDP with either UdR, CdR or AdR. The effect was linear with respect to nucleoside concentration when expressed as a function of CL factor (at 6 h). Similarly, when percent survival, derived from colony-formation experiments, was compared to CL factor derived from alkaline elution of replicate cultures, a linear relation was observed for all cis-DDP and nucleoside combinations.

The mechanisms responsible for this phenomenon have not yet been elucidated. However, it is possible that an excess of nucleosides could lead to their interaction with either free aquated intracellular cis-DDP or with DNA-cis-DDP monoadducts to form amidobridged cis-DDP:nucleosido oligomers in a manner similar to that described for platinum blues. Such putative compounds could have enhanced avidity for DNA, selective ability to shift the binding sites of the monodentation (16) or, more likely, extend the range of the second binding site of the DNA-cis-DDP monoadduct. Thus, many more CL could be formed unimpeded by the short active distance of a single platinum atom, or by steric hindrances between the geometric configuration of the monoadduct and the helical structure of DNA (7,10).

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It has been demonstrated that sulfur nucleophiles such as thiourea can prevent DNA-CL formation and restore biological activity to DNA treated with cis-DDP (8,9). We documented that thiourea not only increases survival of LoVo cells treated with cis-DDP alone, but also in LoVo cells treated with the combination of cis-DDP and ara-C. In both cases, increase in survival was dependent on thiourea concentration and time interval between cytotoxic drug treatment and thiourea administration to the cells. Thiourea had to be added to the cells either simultaneously with cis-DDP or immediately following removal of the drug in order to completely prevent the cytotoxic effect of cis-DDP from being expressed. For the combination of cis-DDP and ara-C, thiourea was able to completely prevent the cytotoxic effect only when added simultaneously with the drug combination. This increase in survival was related to a decrease in DNA-CL, as determined by alkaline elution, both in cells treated with cis-DDP alone and in cells exposed to the combination of cis-DDP and ara-C. Once again, decrease in CL was dependent on thiourea concentration, and the time interval between the additions of drug(s) and thiourea. Thus, in both instances, the time point when thiourea had to be added and the optimal concentration of the sulfur nucleophile were almost identical. These findings suggest that although CL formed by the combination of cis-DDP and ara-C may be more resistant to DNA repair than CL formed by cis-DDP alone, these CL are still sensitive to the rescuing effect of thiourea. Thus, our results support our contention that ara-C induces earlier and more tenacious cis-DDP bidentate DNA-CL. This is reflected by the less

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than 100% survival following thiourea rescue of cells treated with the drug combination, and also by the need for simultaneous treatment of thiourea with ara-C and cis-DDP for maximal decrease of the CL induced by the combination. This could be explained by a larger proportion of cis-DDP:ara-C oligomers that progress from the monoadducted to the bidentate form more rapidly than the cis-DDP-DNA adducts alone, thus becoming impervious to thiourea rescue, at least under our experimental conditions.

Time-dependency of cell survival between additions of cis-DDP and ara-C was established in experiments similar to those described for thiourea. At various time points before harvesting, cells were treated with ara-C (1 mg/ml) for one hour, the drug was removed and cells were incubated in fresh medium. One hour before harvesting, all cells received cis-DDP (10 µg/ml) for one hour. Ara-C had no potentiating effect on cis-DDP toxicity unless given to the cells simultaneously with cis-DDP. When the experiment was reversed so that cis-DDP (10 mg/ml) was added first and ara-C (1 mg/ml) was given one hour before harvesting, a slight potentiating effect was observed even if the drugs were added four hours apart but the optimal toxic effect was obtained when ara-C and cis-DDP were added together. Similar results were obtained when the concentration of cis-DDP was lowered to 5 µg/ml while retaining the concentration of ara-C at 1 mg/ml.

To investigate whether continuous exposure of cells to low concentrations of ara-C and cis-DDP would result in a potentiation of cytotoxicity similar to that noted for higher doses delivered at once, LoVo cells were treated with cis-DDP (1 µg/ml) alone, ara-C (100 µg/ml) alone, or a combination of both agents for continuous periods of up to 24 hours; the selected drug concentrations were 10 times lower than those used in the bolus administration experiments. Following treatment, cells were harvested and analyzed for survival by the clonogenic assay. Ara-C did not induce cytotoxic effects after only 1 hour exposure, but continuous treatment for 24 hours resulted in greater than 97% cell kill. cis-DDP, also had negligible cytotoxic effects after 1 hour treatment but resulted in significant cell kill (about 96%) after 12 hours. Thus, when delivered in continuous treatment at lower concentration the drug combination had an additive rather than a synergistic effect.

Our initial reports concerning the synergistic effects of cis-DDP and ara-C promoted the development of several clinical trials using this drug combination. In most instances, therapeutic design was in clear violation of the vitro data because cis-DDP was used at extremely low bolus or fractionated doses while ara-C was administered over prolonged periods of time. In other cases, both drugs were delivered at very low doses in continuous infusion, or were not administered simultaneously. Thus, most preliminary clinical trials predictably reported a high rate of toxicity with negligible antitumor effect, a result that might be attributed mostly to the defective strategy employed for the combination of these agents. Our studies have clearly demonstrated that the two drugs must be added together in order to obtain the maximal cytotoxic effect. No potentiating effect was obtained if cells were pretreated with ara-C before the exposure to cis-DDP, a finding possibly related to the fact that most cells, including LoVo (11), rapidly deaminate the "faulty" nucleoside. On the other hand, if ara-C was delivered to the cells following treatment with cis-DDP, a slight potentiation of cytotoxicity could still be observed. These results suggest that ara-C did not sensitize the cells to cis-DDP, but that it facilitated the formation of additional DNA-CL by the monoadducted cis-DDP. However, for an effective clinical strategy resulting in maximal therapeutic gain, the rule should be to administer both agents together or within the shortest possible interval between each other. This is even more important in view of the fact that when LoVo cells were continuously exposed to low concentrations of ara-C, cis-DDP or a combination of both drugs for up to 24 hours, an additive rather than a synergistic effect was observed for the combination. As shown before (11), under these conditions ara-C alone did have a marked cytotoxic effect after 24 hours continuous exposure, results that would explain the severe myelotoxicity described in clinical studies that used the ara-C:cis-DDP combination but delivered the antitumor metabolite in prolonged infusion.

It is well known that the cytotoxic activity of the platinum complex is conferred by the leaving anionic ligands. However, the inert ligands can modulate the kinetics of the substitution reaction and/or modify the transport of cis-DDP across the cellular membrane. Therefore, more than 500 platinum analogs have been synthesized by modifying either the leaving or the inert ligands in the hope of finding agents with enhanced activity over that of the parent platinum complex, cis-DDP. Most such compounds failed to show clear superiority over cis-DDP when a broad range of experimental tumors were tested and only a few analogs merited further studies and eventual clinical trials. In a recent report (12), we demonstrated that four such analogs (TNO-6, JM-82, JM-8, and JM-9) showed substantial differences in antiproliferative activity as a function of their particular substitution moieties, and that cis-DDP was still the most active agent followed closely by TNO-6. Data shown on Table 1 demonstrates that while the combination of cis-DDP and ara-C augmented 10-fold the cytotoxic activity of cis-DDP alone, an effect accompanied by an almost 2-fold increase in DNA-CL, every other analog failed to interact in a potentiating manner with the antimetabolite. Furthermore, with the exception of an increased DNA-CL value for the combination of JM-82 and ara-C, all other combinations either retained or actually decreased the CLF value induced by the platinum analog delivered alone. These findings offer further proof of the exquisite sensitivity of the interaction of Pt with its target to the leaving and (possibly the inert) ligands of the complex. Thus, while ara-C interacted with cis-DDP to increase its cytotoxic and DNA-CL activity in the anticipated manner, not one of the analogs interacted with the antimetabolite to induce increased cellular lesions (DNA-CL) or higher cellular death rates. Whether the failure resides in the inability of ara-C to react with the free or monoadducted analogs remains to be elucidated. However, it appears clear that the associated moieties of the Pt coordination complex play a fundamental role in reducing the interaction of the analogs with DNA (as reflected by the decreased CL and cytotoxicity induced by each agent alone) and in totally preventing their interaction with ara-C to yield a potentiating effect. These circumstances demonstrate the meticulous care that must be given to the factors used to administer these antitumor drugs, without which only increased toxicity but no discernible antitumor effect will be observed.

TABLE 2

COMPARISON OF CELL SURVIVAL AND DNA CROSS-LINK FACTOR OF LOVO CELLS TREATED WITH PLATINUM COMPLEXES ALONE OR IN COMBINATION WITH ARA-C.

ANTITUMOR AGENT	CONCENTRATION	ALONE		IN COMBINATION WITH ARA-C	
		% survival* (1SD)	CLF*	% survival* (1SD)	CLF*
ara-C	1,000 μg/ml	30 ± 10	1.06	-	-
cis-DDP	5 μg/ml	50 ± 2.1	2.85	5	5.06
TNO-6	5 μg/ml	35 ± 5.0	2.33	40 ± 4.0	1.62
JM-82	15 μg/ml	37 ± 5.2	1.33	33 ± 1.5	2.25
JM-8	25 μg/ml	80 ± 7.2	1.41	80 ± 6.4	1.00
JM-9	25 μg/ml	80 ± 12.0	1.34	75 ± 5.3	1.37

*Average of at least 2 independent determinations.

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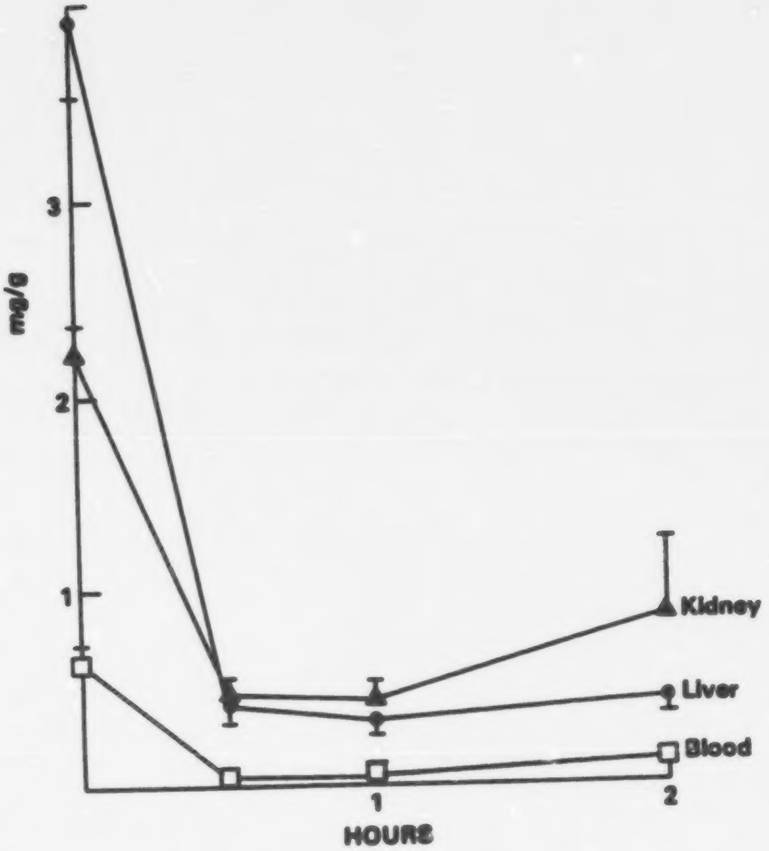
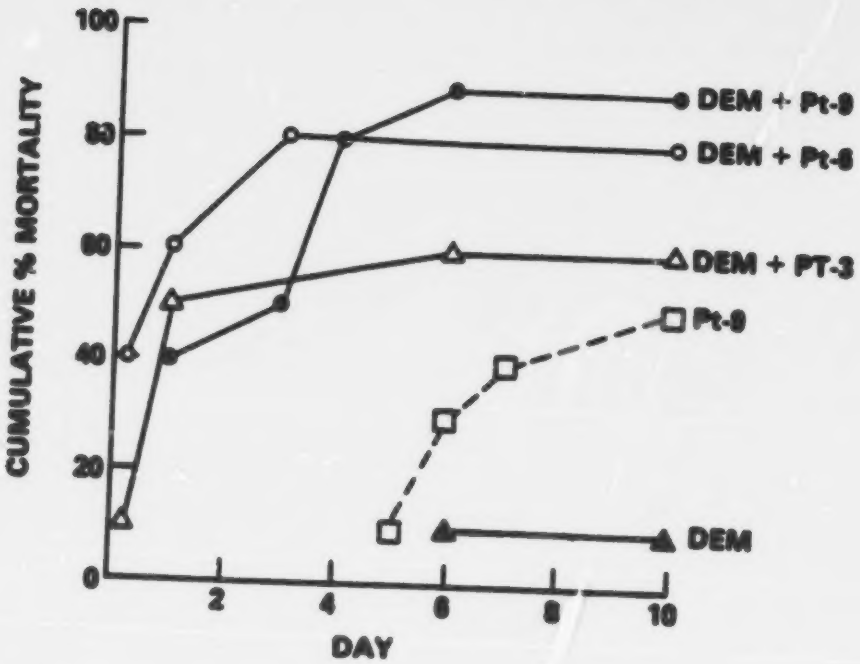
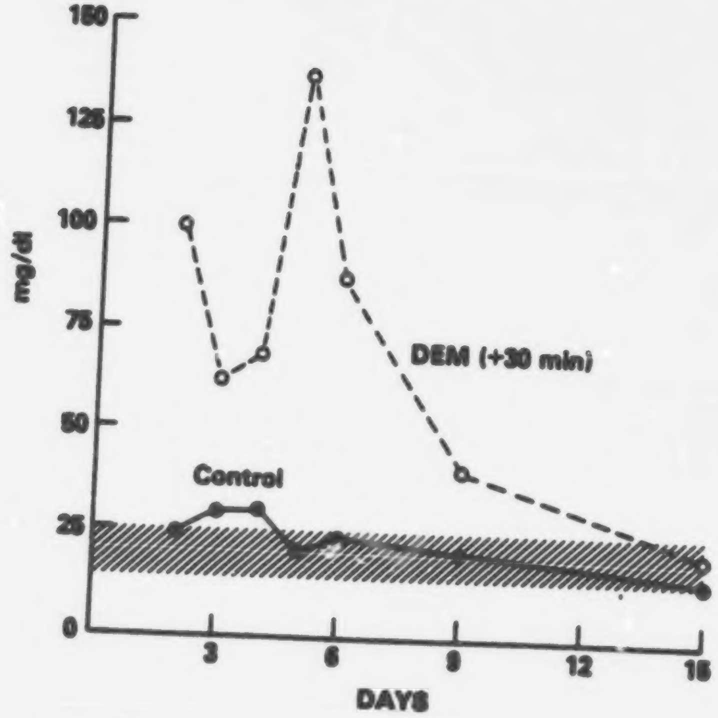
TISSUE SULFHYDRYLS AFTER 1mg/kg DEM



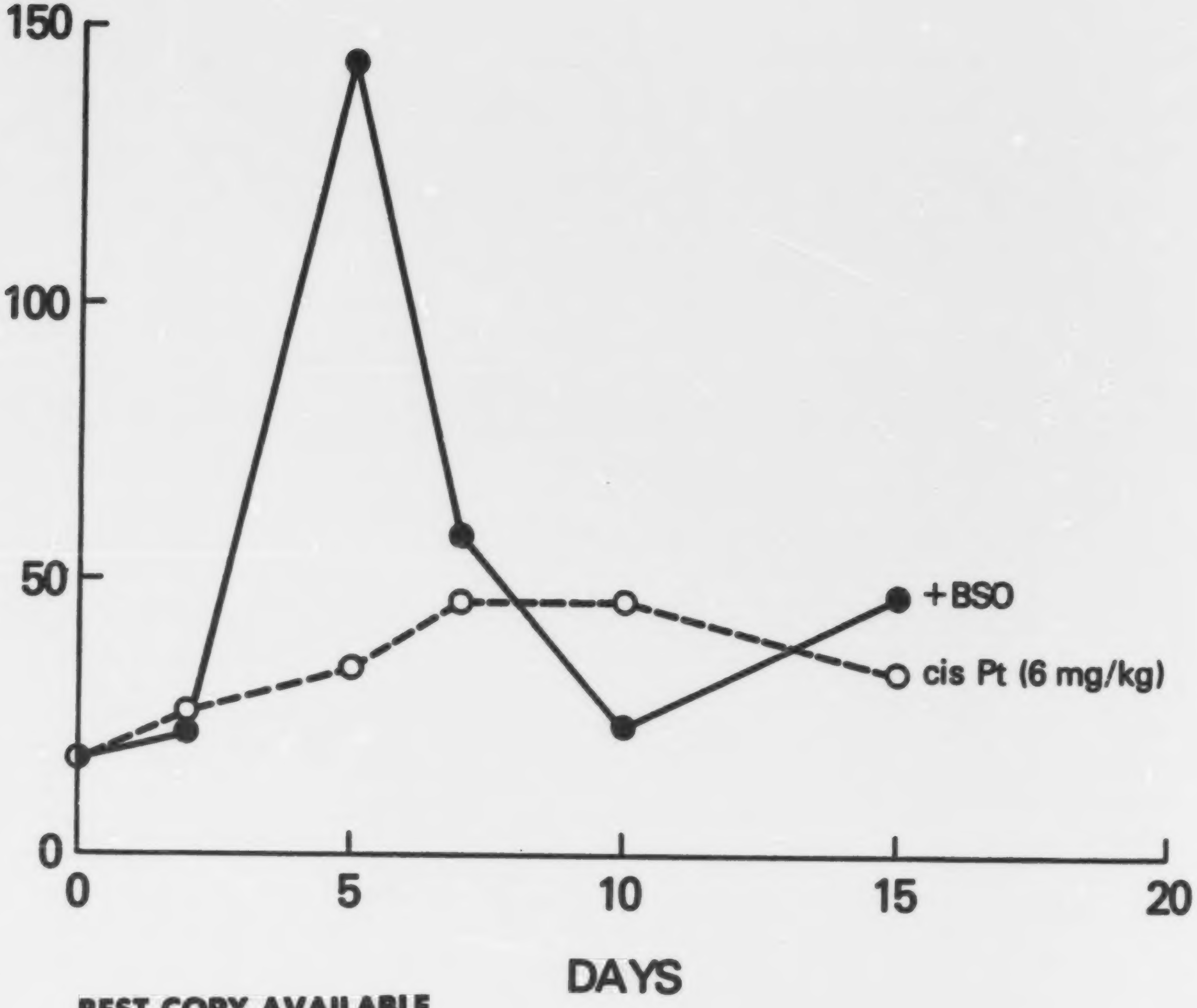
Cisplatin Mortality in DEM-Pretreated (30') Rats



BUN Values after 3 mg/kg *Cisplatin*



BSO PRETREATMENT AND BUN ACTIVITY



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DEM (1 mg/kg) EFFECT ON Pt DISTRIBUTION AFTER 8 mg/kg CISPLATIN (mg/g)

	Control	DEM
5 MIN		
Kidney	3.74	3.44
Liver	0.41	0.41
Lung	0.70	0.71
Colon	0.47	0.62*
Duodendum	0.40	0.50
Plasma	0.76	0.92*
24 HOUR		
Kidney	1.46	2.08*
Liver	0.31	0.45
Lung	0.13	0.23*
Colon	0.16	0.23
Duodendum	0.12	0.22*
Plasma	0.07	0.16*

1 hr: no differences   6 hr: Liver 0.25/0.55

* = $p \leq .05$

DEM (1 ml/kg) EFFECT ON Pt DISTRIBUTION IN KIDNEY (ng Pt/mg protein)

	Control	DEM
2 MIN		
Mitoch	111 ± 23	187 ± 39*
Micro	124 ± 3	133 ± 17
Cytosol	505 ± 47	631 ± 59*
1 HOUR		
Mitoch	104 ± 20	150 ± 15*
Micro	91 ± 2	123 ± 3*
Cytosol	217 ± 41	254 ± 35
24 HOUR		
Mitoch	700 ± 44	815 ± 70*
Micro	204 ± 30	299 ± 14*
Cytosol	105 ± 6	145 ± 28*

* $p \leq .05$

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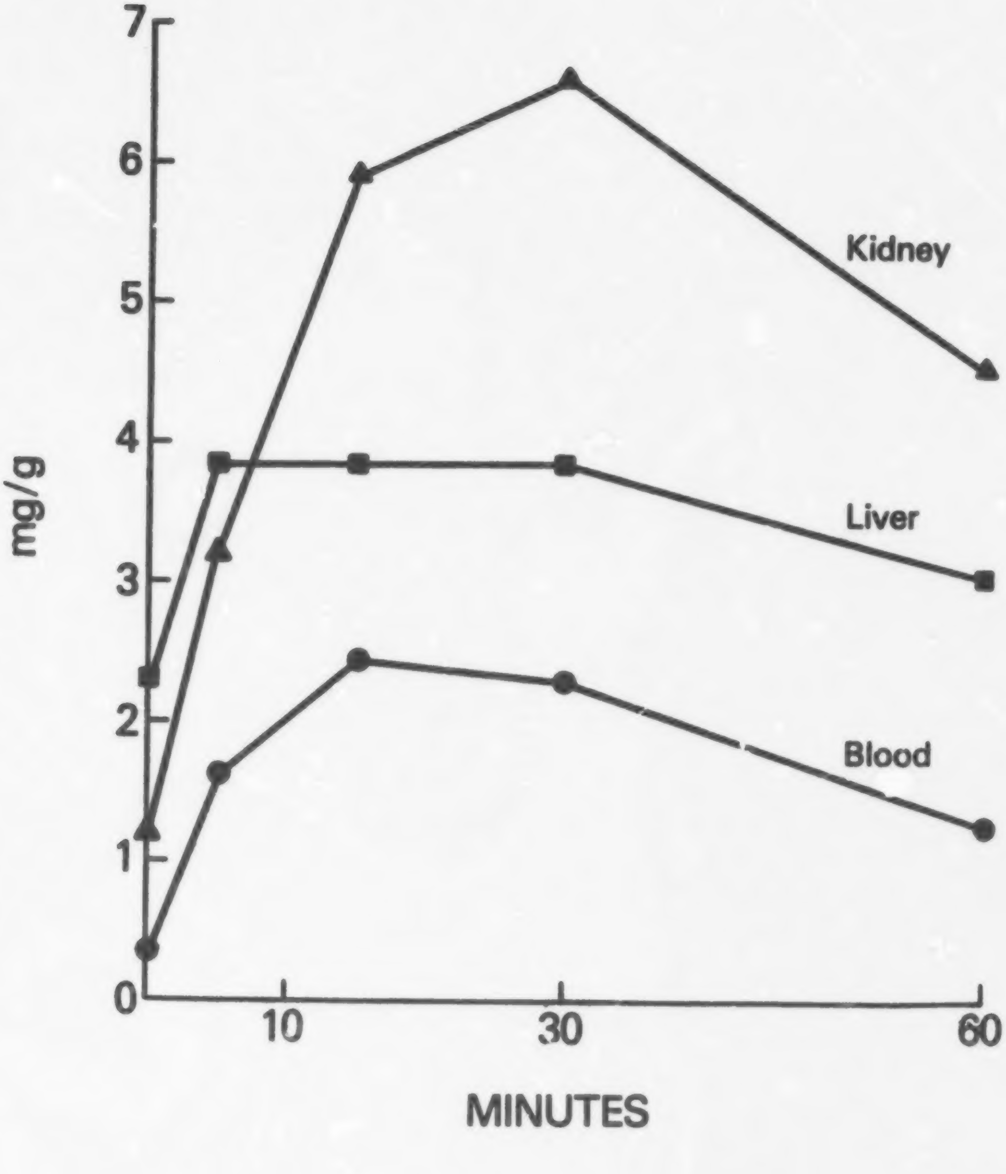
TISSUE SULFHYDRYL CONTENT AFTER 500mg GSH IP



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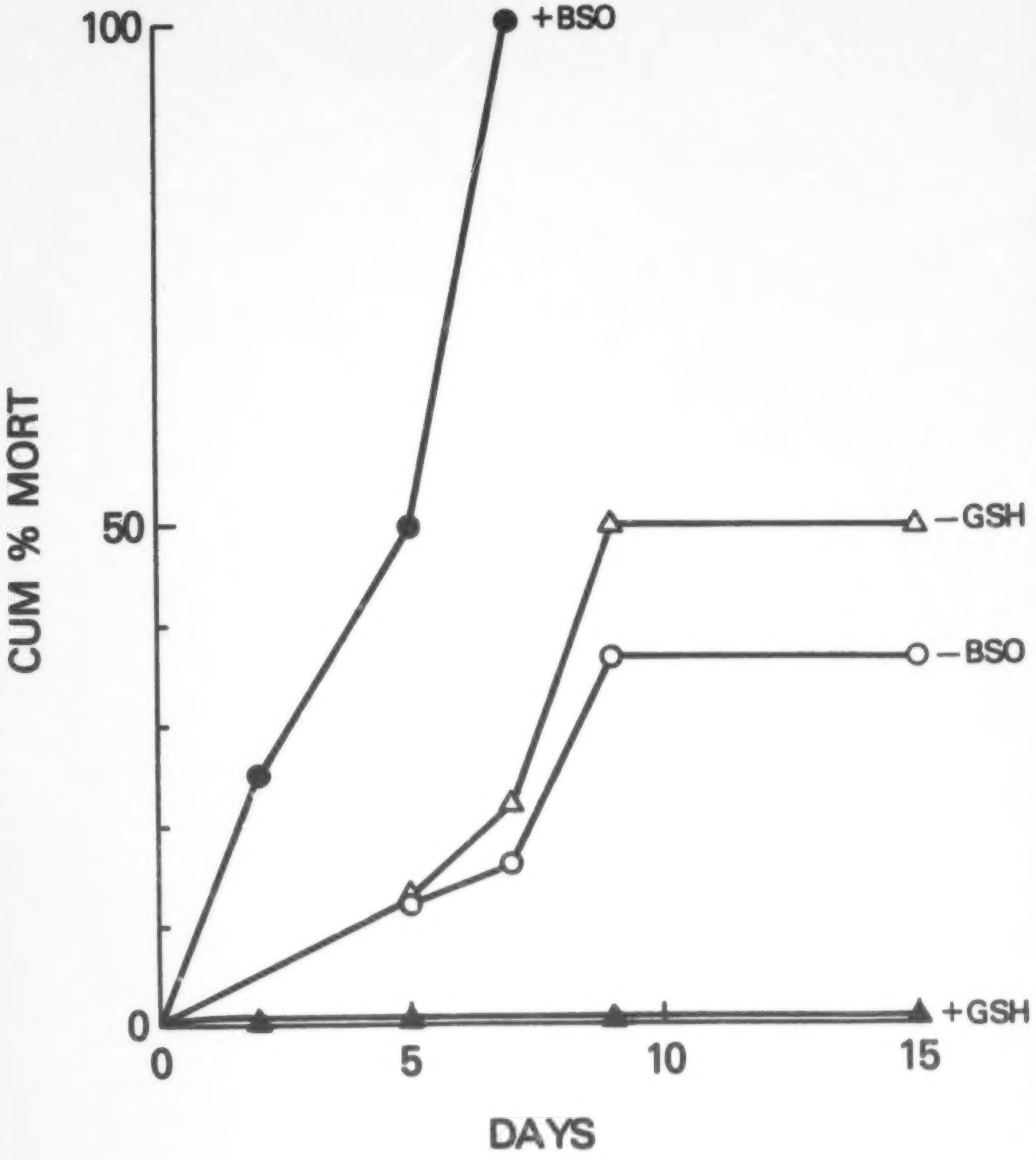
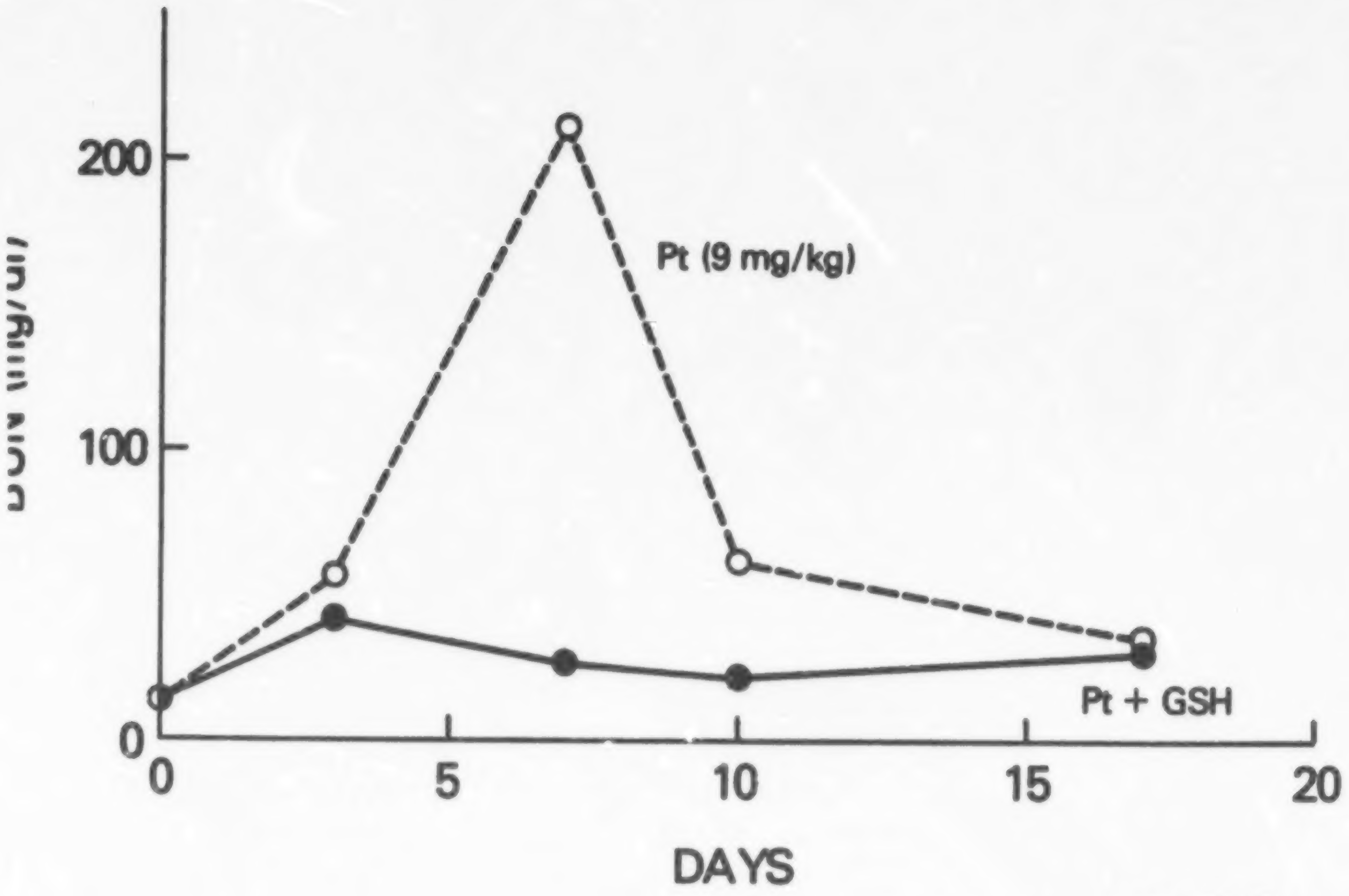
PRETREATMENT EFFECT ON MORTALITY
cis Pt (9 mg/kg)



GSH PRETREATMENT AND BUN ACTIVITY



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GSH (1 g/kg) EFFECT ON Pt DISTRIBUTION AFTER 8 mg/kg CisPt (T:P)

	Control	GSH
1 Hour		
Kidney	11.3	8.60 *
Liver	2.51	2.61
Lung	2.15	1.94 *
Duodenum	1.99	1.78
24 Hour		
Kidney	27.5	30.0
Liver	5.04	4.96
Lung	2.69	1.55 *
Duodenum	1.95	1.88

5 min: no differences

6 hr: Kid 24.4/15.5; Lg 2.5/2.0

* $P \leq .05$

EFFECT OF GSH ON PLATINUM SUBCELLULAR DISTRIBUTION (dpm/mg)

		NUC	MITO	MICRO	CYTO
5 Minutes	−GSH	123	68	52	690
	+GSH	110	85*	55	841*
1 Hour	−GSH	88	74	63	214
	+GSH	75*	60*	42*	326*
6 Hours	−GSH	81	117	77	261
	+GSH	81	138*	65*	322*
24 Hours	−GSH	173	285	93	378
	+GSH	133*	194*	66*	328

*P< .05

EFFECT OF GSH PRETREATMENT ON cisPt ANTITUMOR ACTIVITY

Treatment	$\overline{x}$ Survival (days)
None	14
GSH (1g/kg)	16
cisPt (10mg/kg)	23
Pt + GSH	22

2x10^6 P388 cells SC; GSH ip 15 min before cisPt iv; tumor 24hr before drugs

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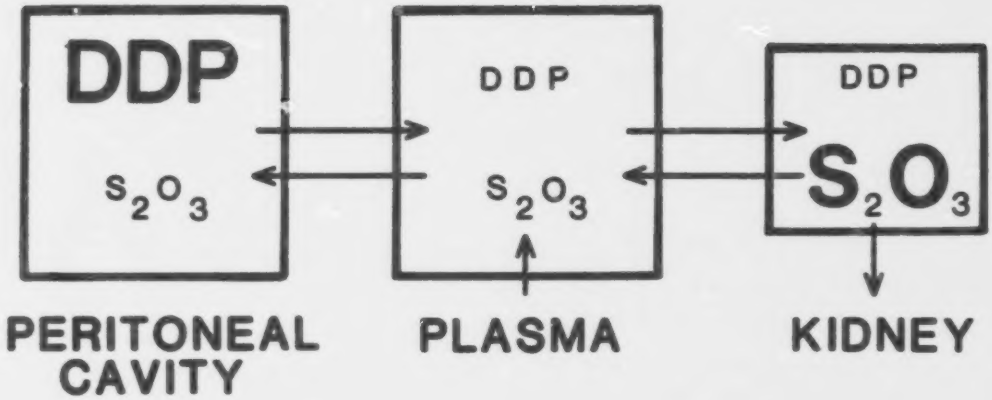
SUMMARY

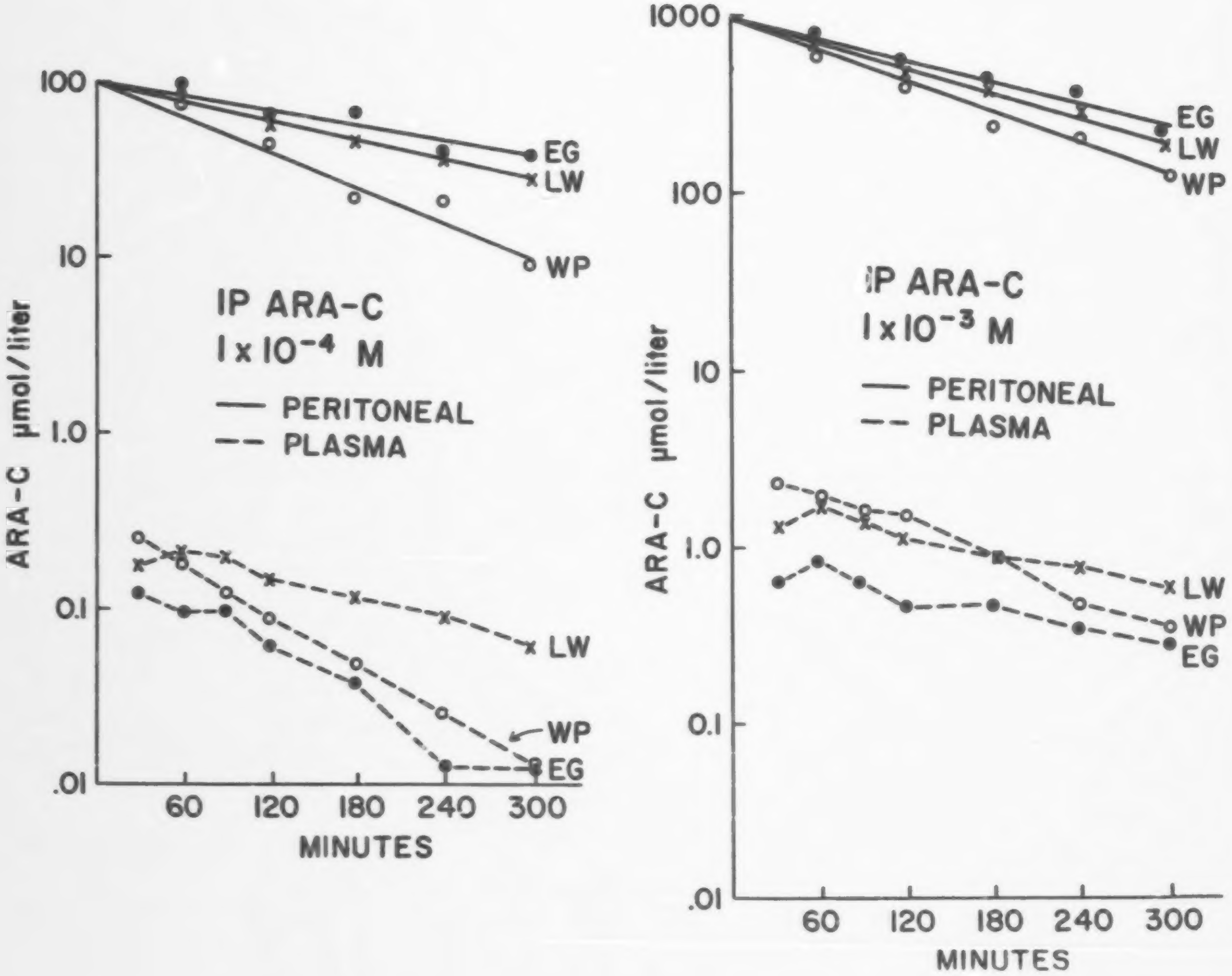
1. Decreases in endogenous GSH levels by DEM or BSO produce increases in CisPt toxicity
2. DEM (1 ml/kg) produced increases in plasma Pt concentration but no consistent changes in Pt organ distribution
3. DEM produced significant increases in Mito, Micro, Cyto Pt concentrations beginning 2 min after CisPt and lasting at least 24 hr
4. Increases in endogenous levels of GSH by GSH administration produce decreases in CisPt toxicity
5. GSH produced decreases in plasma Pt concentration but no consistent changes in Pt organ distribution
6. GSH produced consistent increases in Cyto Pt concentrations throughout the first 6 hr, while Micro Pt content decreased, and variable changes were seen in Mito and Nuc Pt concentrations

FIGURE 1


DDP
S_2O_3
DDP
S_2O_3
DDP
S_2O_3
PERITONEAL CAVITY
PLASMA
KIDNEY

Principles of intraperitoneal chemotherapy. Direct instillation of many chemotherapeutic agents results in very high local concentrations in the peritoneal cavity relative to their concentration in the systemic circulation.

FIGURE 2



Pharmacokinetics of intraperitoneally administered cytarabine. When cytarabine is administered intraperitoneally the local concentration is 300 to 1000-fold higher than the concentration in the plasma.

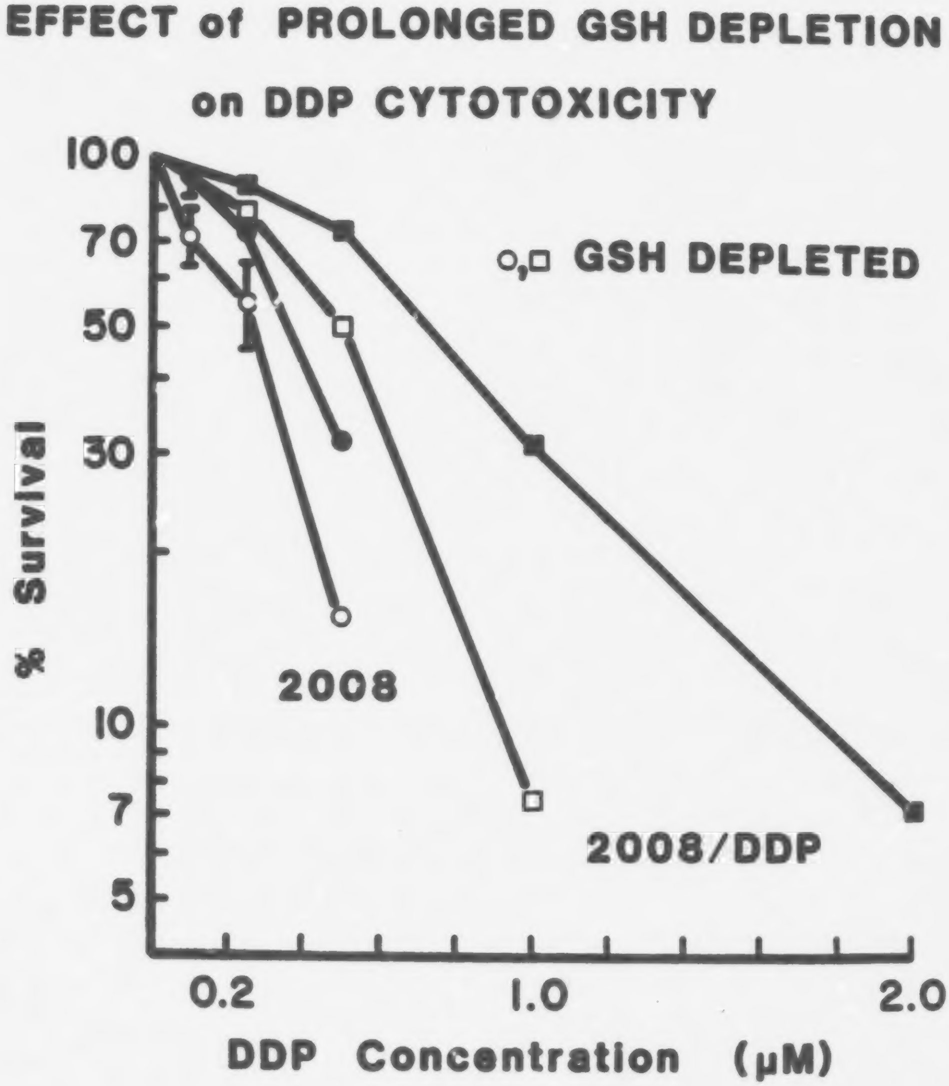
Methods for Analysis of Drug Interaction

Product of the Surviving fraction
Isobologram analysis
Median Effect analysis

FIGURE 3

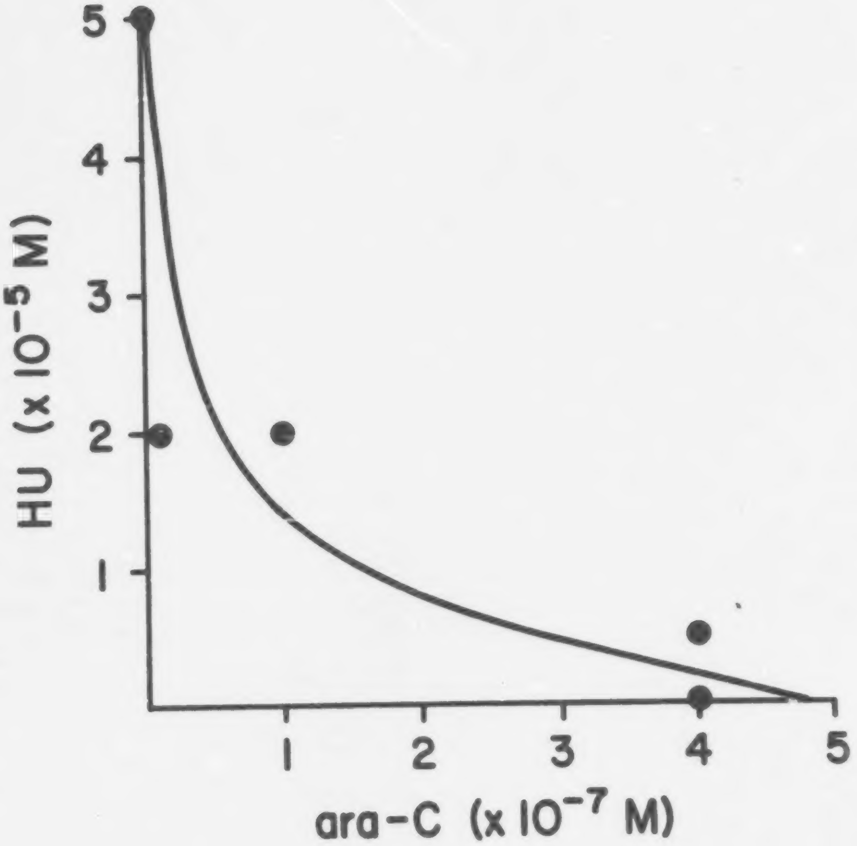
Methods for analysis of drug interaction. Currently three methods are available. The product of the surviving fraction method is applicable only when dose response curves are log linear. Isobologram analysis is more generally applicable, but requires a large number of experimental data points. Median effect analysis is the most general, but is sensitive to small degrees of interexperiment variation.

FIGURE 4



Determination of synergy by the product of the surviving fraction method.

FIGURE 5



Determination of synergy by isobologram analysis.

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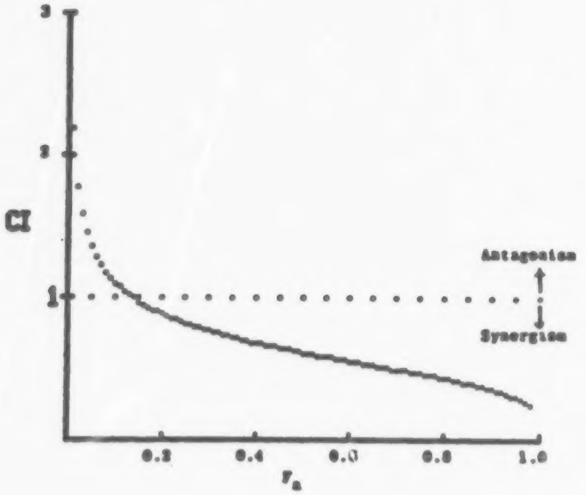
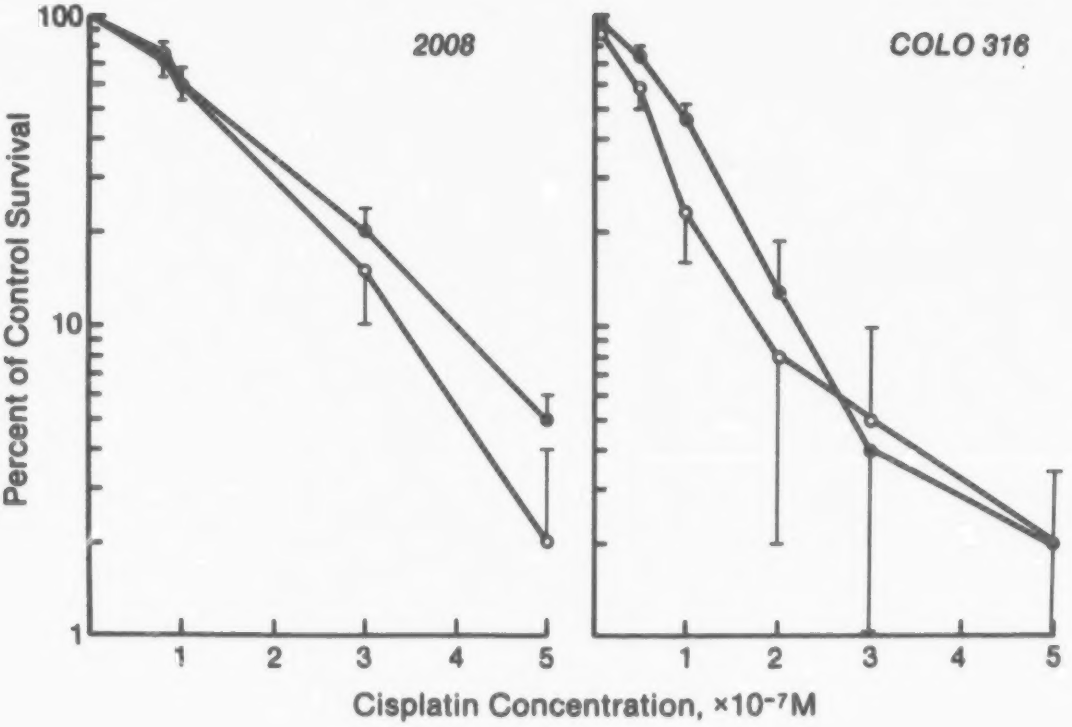
FIGURE 6



Determination of synergy by median effect analysis.

FIGURE 8



Lack of synergy between cisplatin and cytarabine when cells are exposed only for 4 hours.

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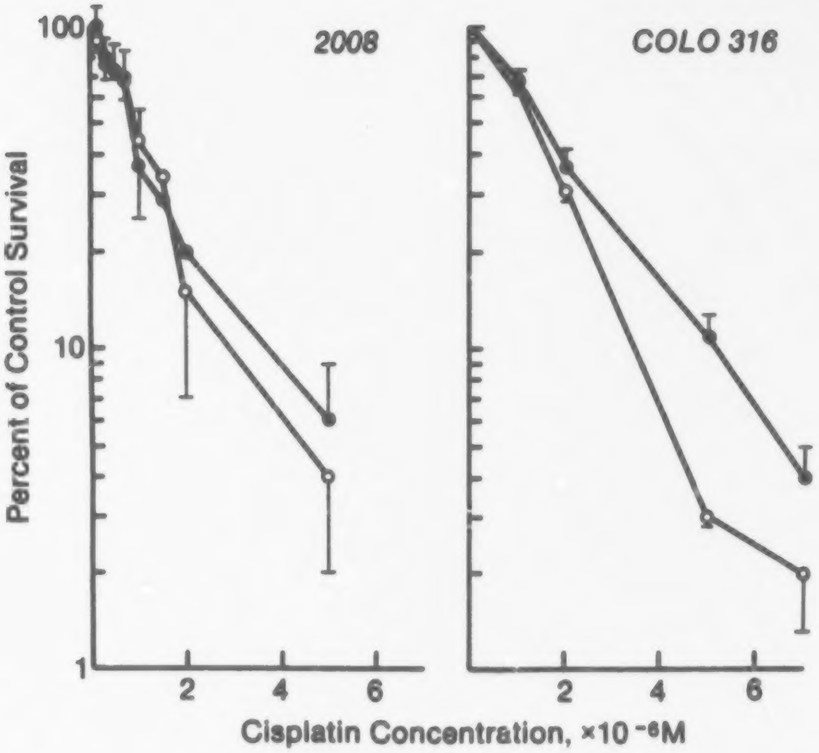
FIGURE 7



Lack of synergy between cisplatin and cytarabine when both drugs are present in the culture throughout the whole period of colony formation.

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FIGURE 9

Median effect analysis of the interaction between cisplatin and etoposide showing minimal degrees of synergy at low drug concentrations, and some antagonisms at high drug concentrations.

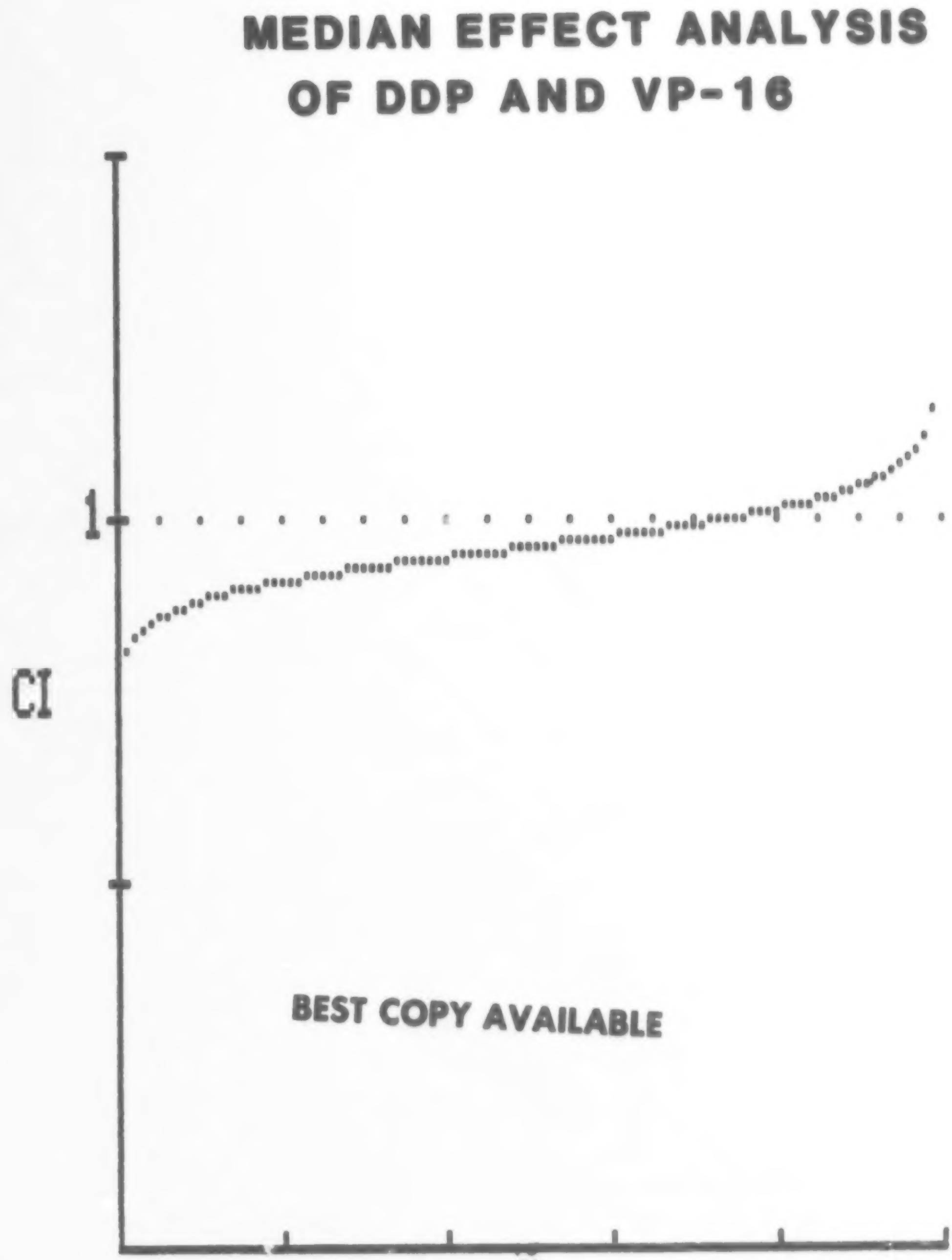

FIGURE 10

The effect of TPA on differentiation of human ovarian carcinoma cells in culture.

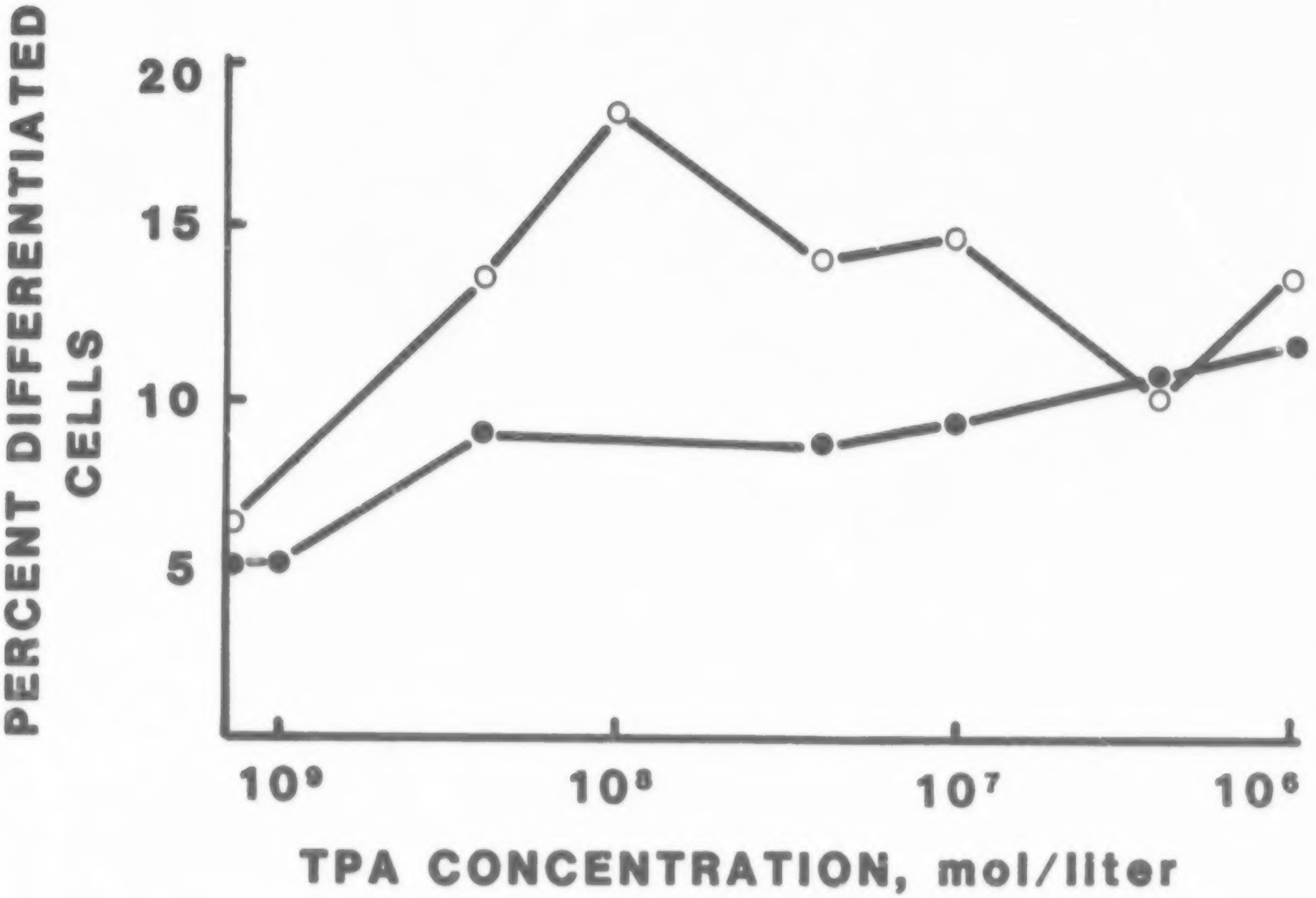

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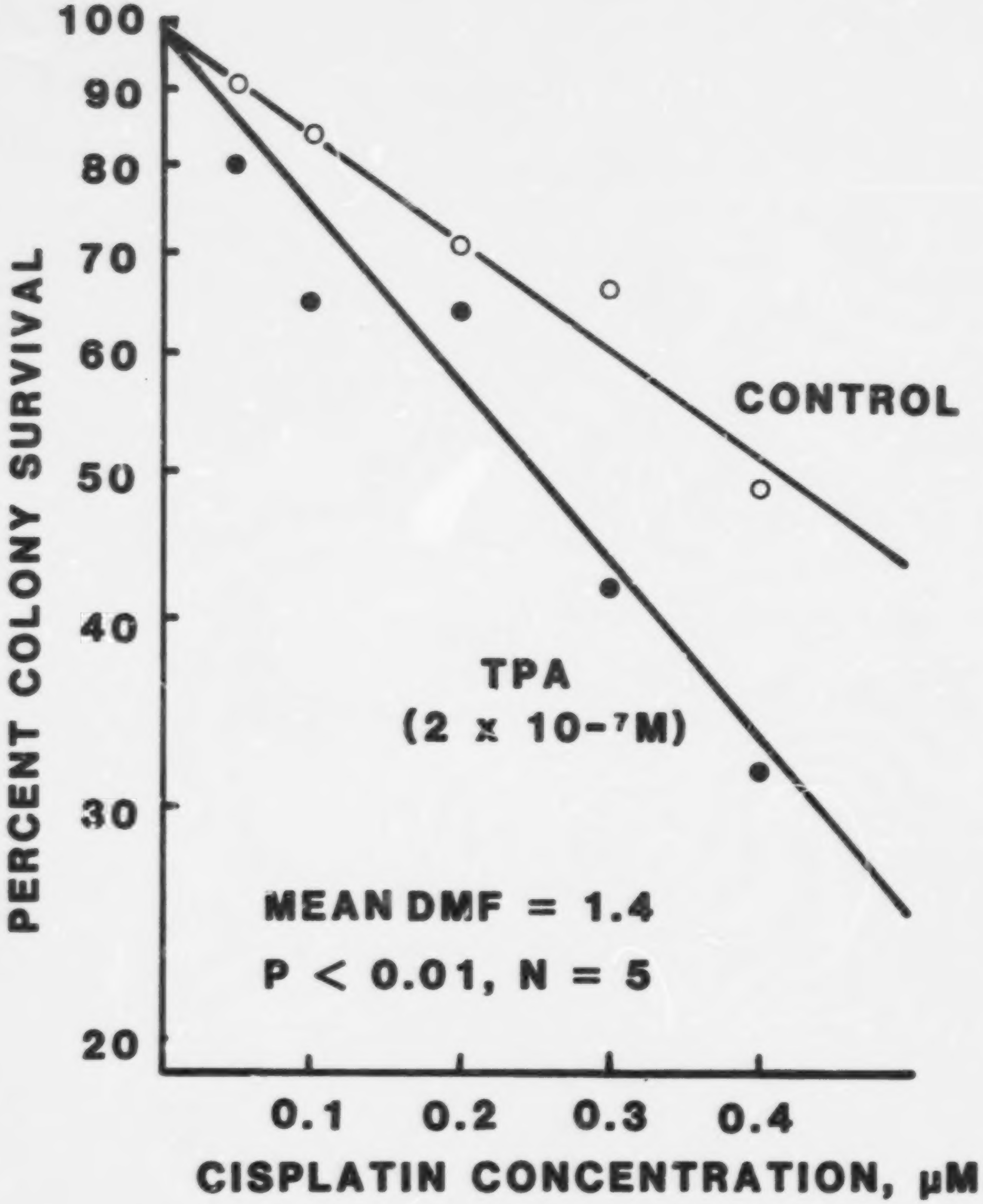
FIGURE 11



The ability of TPA to increase sensitivity of human ovarian carcinoma cells to cisplatin.

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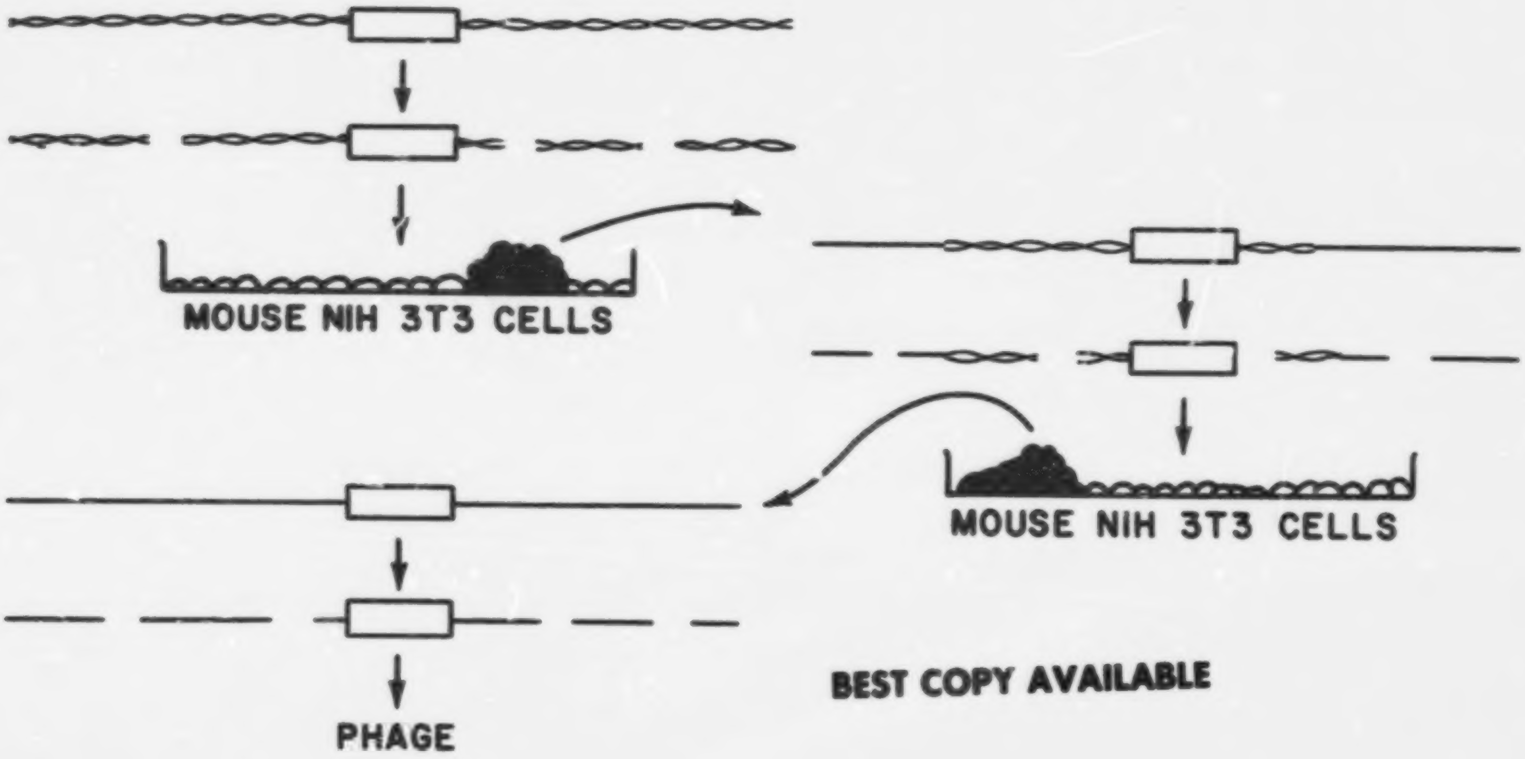
FIGURE 12

TRANSFECTION


MOUSE NIH 3T3 CELLS
MOUSE NIH 3T3 CELLS
PHAGE

Principles of the transfection technique for introduction of activated oncogenes into eucaryotic cells.

FIGURE 13

Table 1. Sensitivity of parental NIH 3T3 cells and clones containing inserted activated H-ras.

	IC$_{50}$ Concentration (x 10^{-8}M)		
Cell type	Cisplatin	Doxorubicin	Fluorouracil
Parental 3T3	40.5	13.9	104
Clone A	205	7.6	182
Clone B	60	4.5	87
Clone C	96	7.6	70
Clone D	55	8.5	104
Clone E	31	8.4	42
Clone F	51	9.6	72
Clone G	41	6.1	49
Clone H	46	15.3	65
Clone I	17	7.6	21
Clone K	25	16.0	130

Insertion of an activated form of the H-ras gene does not change the sensitivity to 3 categories of anticancer agents.

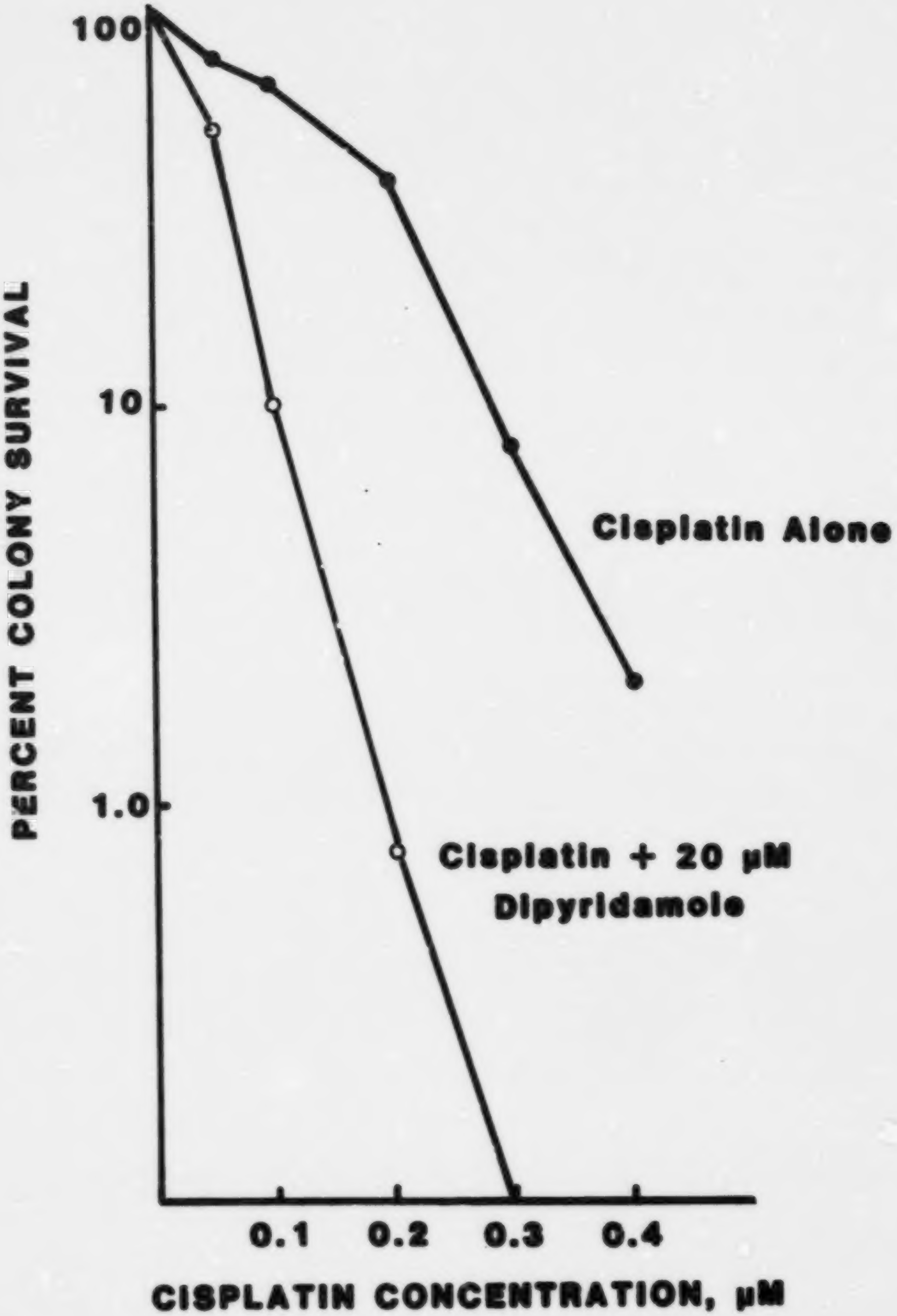
Drugs Screened by "Median Effect Analysis" for Synergy with Cisplatin

Bleomycin
5-fluorouracil
Verapamil
Melphalan
Quinidine
Putrecine
Etoposide
Cytarabine

FIGURE 14

None of the drugs listed showed significant interaction with cisplatin by median effect analysis.

FIGURE 15



The ability of dipyridamole to sensitize human ovarian carcinoma cells to cisplatin.

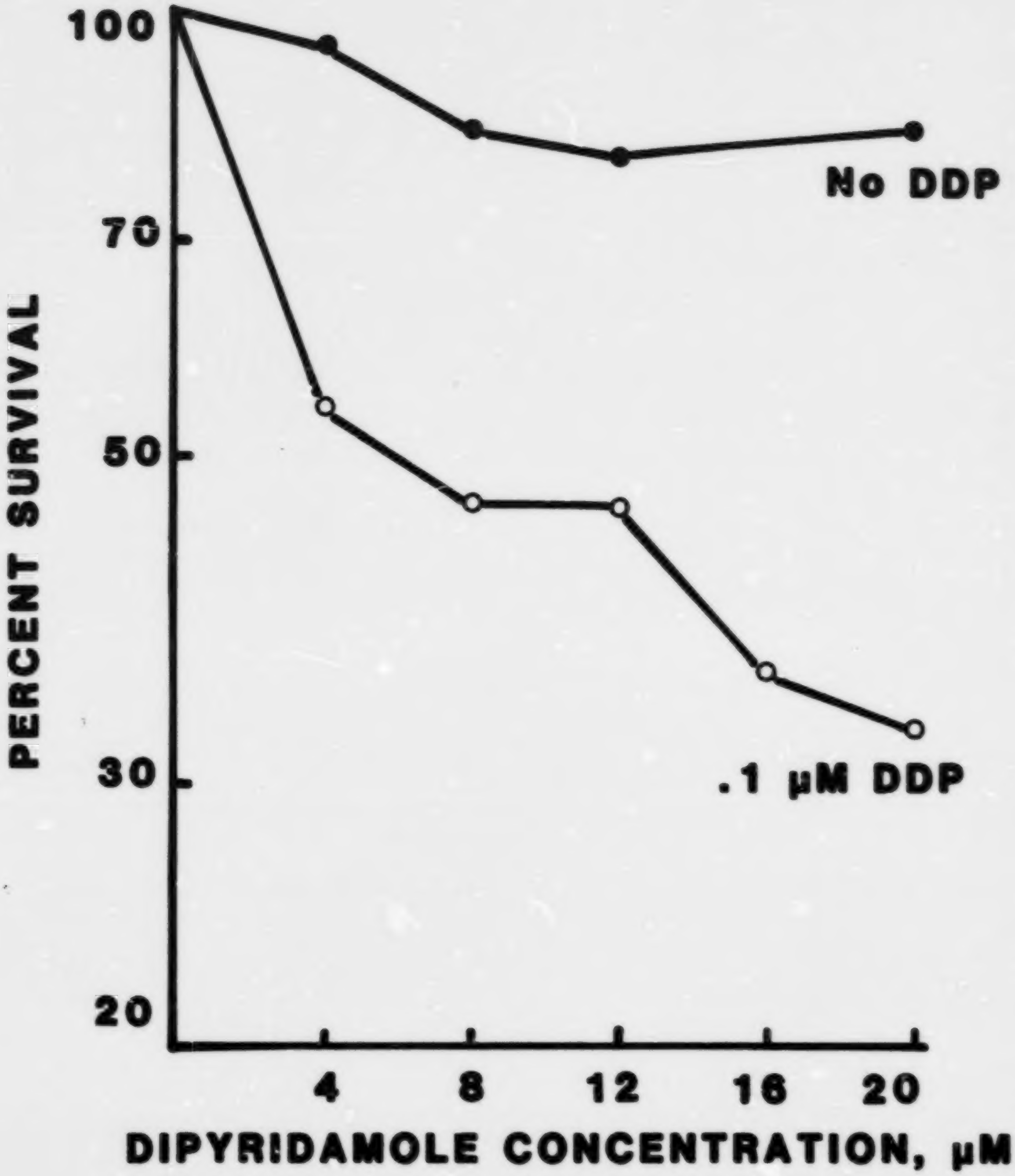


FIGURE 16

Concentration dependence of the ability of dipyridamole to enhance the sensitivity of human ovarian carcinoma cells to cisplatin.

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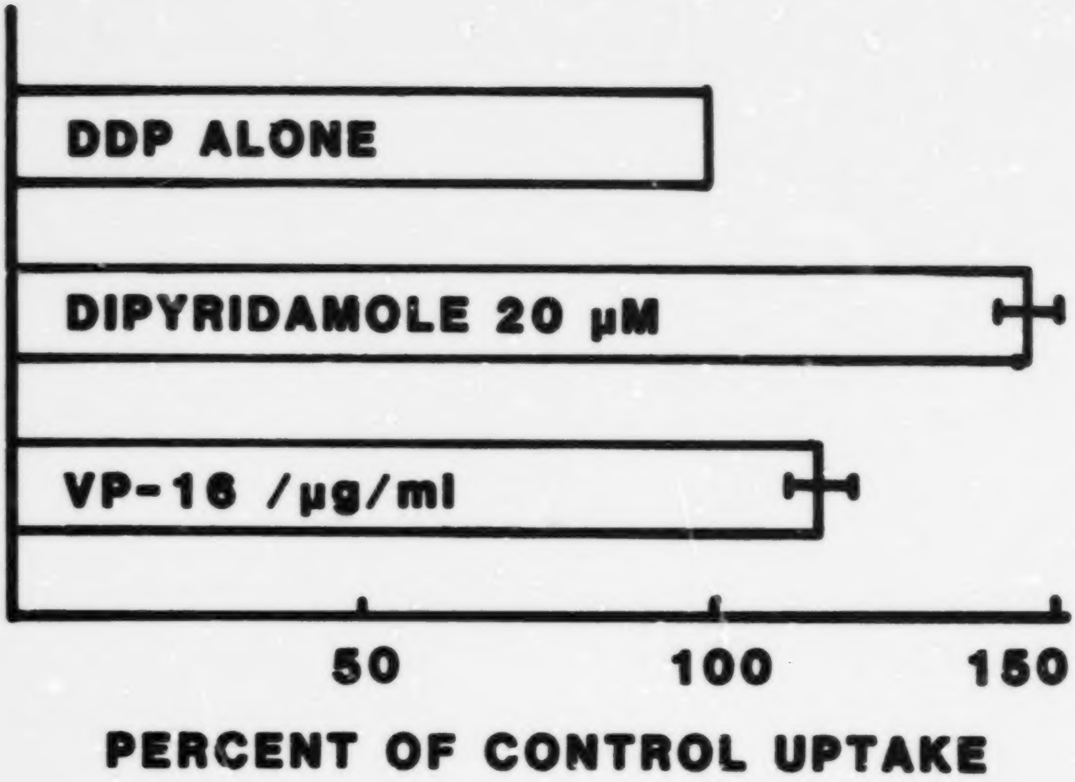
FIGURE 17



Dipyridamole increases the uptake of cisplatin into human ovarian carcinoma cells.

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NORRIS HOSPITAL

A RANDOMIZED PHASE I STUDY OF SHORT-TERM INFUSION OF HIGH-DOSE 5-FU WITH OR WITHOUT PALA IN PATIENTS WITH ADVANCED COLORECTAL AND PANCREATIC CANCERS.

IN THE DESIGN OF THE PHASE I STUDY. I INCORPORATED THE EXACT REQUIREMENTS OF THE LABORATORY OBSERVATION.

(1) ADMINISTER PALA AS I.V. PUSH AT LEAST 24 HRS BEFORE 5-FU (BASED ON OUR OBSERVATION OF THE INHIBITION OF LEUKOCYTE ATCASE BY PALA).

(2) USE OF A MODULATING DOSE OF PALA, NOT A THERAPEUTIC DOSE OF THE AGENT.

(3) EXPOSURE TO 5-FU FOR AT LEAST 24 HRS OF CONTINUOUS TREATMENT AT MAXIMALLY TOLERATED DOSE (MTD) OF THE AGENT (THE LATTER WAS NOT KNOWN).

(4) WEEKLY TREATMENT WITH EITHER THE SINGLE AGENT OR THE COMBINATION OF THE AGENTS.

OBJECTIVES

1) TO DETERMINE THE MAXIMUM TOLERATED DOSE (MTD) OF 5-FU, AND THE COMBINATION OF PALA AND 5-FU IN THE MANNER ADMINISTERED.

2) TO DETERMINE THE QUALITATIVE AND QUANTITATIVE TOXICITY OF THE TWO TREATMENT ARMS.

3) TO ASSESS THE CLINICAL RESPONSE IN THE TWO TREATMENT ARMS.

4) TO UNDERGO PHARMACOKINETIC STUDIES.

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Norris Hospital
July 1986

PHASE I STUDY OF 5-FU VS 5-FU AND PALA
PATIENT ELIGIBILITY

1) HISTOLOGICALLY PROVEN COLON, RECTAL AND PANCREATIC CANCERS.

2) INFORMED CONSENT.

3) LIFE EXPECTANCY OF MORE THAN 12 WEEKS.

4) KARNOFSKY GREATER THAN 40%.

5) MEASURABLE DISEASE.

6) NO PREVIOUS CHEMOTHERAPY.

7) WBC > 3000; PLATELETS $> 100,000$.

8) SERUM CREATININE < 2.0 MG%.

9) SERUM BILIRUBIN < 2.0 MG%.

10) NORMAL SERUM ELECTROLYTES AND GLUCOSE.

TREATMENT ASSIGNMENT

ARM	AGENT	DOSAGE/ROUTE
I	PALA	250 MG/M2 I.V. (15 MINS)
	5-FU	750 MG/M2 I.V. (OVER 24 HRS.)
II	5-FU	1000 MG/M2 I.V. (OVER 24 HRS.)

WEEKLY TREATMENT SCHEDULES

ESCALATION: PALA DOSE WILL REMAIN FIXED AT 250 MG/M2

ARM I	LEVEL 2: 5-FU	1000 MG/M2
	" 3: "	1300 MG/M2
5-FU/	" 4: "	1600 MG/M2
PALA	" 5: "	2000 MG/M2
	" 6: "	2600 MG/M2
	" 7: "	3400 MG/M2
ARM II	LEVEL 2: 5-FU	1300 MG/M2
	" 3: "	1600 MG/M2
5-FU	" 4: "	2000 MG/M2
ALONE	" 5: "	2600 MG/M2
	" 6: "	3400 MG/M2
	" 7: "	5200 MG/M2

PHASE I - PALA/5-FU VS 5-FU

PATIENTS CHARACTERISTICS

NO. PATIENTS		44
NO. EVALUABLE PATIENTS		42
NO. OF COURSES		804
NO. OF EVALUABLE COURSES		790
M/F		23/21
AGE: MEDIAN		54
RANGE		24-78
DIAGNOSIS:		
COLON	19	
RECTAL	11	
PANCREAS	14	
PRIOR TREATMENT:		
CHEMO. OR RADIATION	0	
OFF STUDY	35	
ON STUDY	9	
CYCLES/PATIENT MEDIAN 19	(4-52)	

PHASE I STUDY PALA/5-FU VS 5-FU

HEMATOLOGICAL TOXICITY
5-FU ALONE ARM

5-FU DOSE	NO EVAL	NADIR GRANULO	NADIR
3400 MG/M2	COURSES	CYTES X10 3	PLATELETS
(LEVEL 6)	2	0.5	60,000

#DIC SCREEN +VE

PHASE I STUDY PALA/5-FU VS 5-FU

HEMATOLOGICAL TOXICITY
PALA/5-FU ARM

5-FU DOSE MG/M2	NO EVAL COURSES	NADIR GRANULO-CYTES X 10 3	NADIR PLATELETS
3400 (LEVEL 7)	7	.9	80,000

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PHASE I STUDY OF PALA/5-FU VS 5-FU
NON-HEMATOLOGICAL TOXICITY

DOSE 5-FU MG/M2	750	1000	1300	1600	2000	2600	3400
EVAL	(70)	(154)	(126)	(193)	(180)	(40)	(16)
NO. OF PATIENTS	2	3	6	8	15	6	5
TOXICITY							
ANOREXIA	-	1	2	1	6	4	3
NAUSEA							
MILD	-	1	3	4	6	4	4
SEVERE	-	-	-	-	-	2	2
VOMITING	-	1	2	2	5	3	2
STOMATITIS							
MILD	-	-	-	2	4	3	-
SEVERE	-	-	-	-	-	-	1
DIARRHEA	-	-	1	3	4	2	3
WEAKNESS	-	1	1	2	2	2	1
ATAXIA	-	-	1	2	2	3	4
LOSS OF HAIR							
SCALP	-	-	-	2	2	2	1
EYELASHES	-	-	-	-	1	1	-
PEELING							
HANDS	-	1	2	3	2	2	1
FEET	-	-	-	-	2	2	-

PHASE I STUDY PALA/5-FU VS 5-FU

THE MAJOR DOSE-LIMITING TOXICITY IS ATAXIA WHICH IS SEEN IN FOUR OUT OF FIVE PATIENTS WHO WERE TREATED AT 5-FU DOSE OF 3400 MG/M2. ATAXIA WAS SEEN AT 12-16 WKS WITHIN THE THERAPY AT THE TIME THAT THE CLINICAL RESPONSE HAD BEEN ACHIEVED.

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A RANDOMIZED PHASE I STUDY OF SHORT-TERM INFUSION OF HIGH-DOSE 5-FU WITH OR WITHOUT PALA IN PATIENTS WITH ADVANCE COLORECTAL AND PANCREATIC CANCERS

	LEVEL	NO. PATIENTS	CR	PR	ST	PROG	UNEVALU
5-FU-PALA	1	2	1			1	
5-FU		1				1	
5-FU-PALA	2	2				2	
5-FU		3			2	1	
5-FU-PALA	3	3			1	1	1
5-FU		5		1	1	1	2
5-FU-PALA	4	3		2	1		
5-FU		7			1	5	1
5-FU-PALA	5	8		4		3	1
5-FU		3				3	
5-FU-PALA	6	3		1	1	1	
5-FU		3				3	
5-FU-PALA	7	2	1	1			
5-FU		-					

PHASE I STUDY OF PALA/5-FU VS 5-FU

CONCLUSIONS

1- SEVERE TOXICITY WITH MYELOSUPPRESION. GASTRO-INTESTINAL AND NEUROLOGICAL SYMPTOMS AND SIGNS HAVE BEEN OBSERVED IN PATIENTS TREATED WITH 5-FU AT DOSE OF 3400 MG/M2.

2- SIGNIFICANT CLINICAL RESPONSE IN FORM PARTIAL RESPONSE HAS BEEN SEEN IN TREATMENT LEVELS 4 AND 5. IT IS INTERESTING TO NOTE THAT THE RESPONSES WERE IN THE COMBINATION ARM. AVERAGE TIME TO ACHIEVE PR IN LEVELS 4 AND 5 IS EIGHT WEEKS. THIS PERIOD WAS SUBSTANTIALLY SHORTENED IN LEVELS 6 AND 7, IN WHICH PR WAS ACHIEVED IN FOUR WEEKS. TWO OUT OF TWO PATIENTS AT LEVEL 7 HAVE RESPONDED (1) PANCREATIC CANCER, WITH A MEASURABLE PANCREATIC HEAD MASS, PR; (2) COLONIC CANCER, WITH LIVER METASTASES, CR.

3- MTD FOR 5-FU EITHER ALONE OR IN COMBINATION HAS BEEN REACHED _ 5-FU DOSE OF 2600 MG/M2 I.V. INFUSION OVER 24 HRS Q WEEKLY IS RECOMMENDED FOR PHASE II STUDIES. HOPEFULLY RANDOMIZATION WILL BE OBSERVED, AND A CROSS-OVER WILL BE DESIGNED IN THE FUTURE STUDIES.

4- IT IS HIGHLY PROBABLE THAT A 5-FU/PALA COMBINATION MAY BE EFFECTIVE IN OTHER SITES PRESENTING WITH HISTOLOGICAL PICTURE OF ADENOCARCINOMA.

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TREATMENT PROGRAM

DIPYRIDAMOLE 50 MG/M^2 P.O. Q6H

(BEGINNING 1 WEEK BEFORE PALA ADMINISTRATION)

CONTINUED WHILE PATIENT REMAINED ON PROGRAM

PALA DOSE DELIVERED IV OVER 30 MINUTES

INITIAL DOSE 500 MG/M^2

INCREASED IN 300-500 MG/M^2 INCREMENTS

PALA ADMINISTERED AT 21 DAY INTERVALS

TUMOR TYPES TREATED

	NUMBER OF PATIENTS
LUNG	17
COLORECTAL	14
SARCOMA	10
ADENOCARCINOMA - UNKNOWN PRIMARY	5
PANCREAS	4
OVARY	3
ALL	1
OTHER SOLID TUMORS	12

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PATIENT CHARACTERISTICS

	NUMBER OF PATIENTS
TOTAL	65
male/female	44/21
AGE IN YEARS: MEDIAN 63 (RANGE: 29-83)	
ECOG PERFORMANCE STATUS	
1,2/3	50/15
PRIOR TREATMENT:	
NO PRIOR THERAPY	4
SURGERY AND/OR RADIATION ONLY	6
RADIATION THERAPY	32
CHEMOTHERAPY	55
NUMBER OF PREVIOUS AGENTS:	
$\leq 4 / > 4$	38/17

TOXICITY OF PALA/DIPYRIDAMOLE PROGRAM

DOSE (MG/M^2)	#COURSES	RASH	MUCOSITIS	DIARRHEA/ ABDOMINAL CRAMPING
< 3000	37	0	1	1
3000	23	2	3	4 (2)
3300	21	2 (1)	1 (1)	1 (1)
3600	12	0	3	6
3900	10	2	1	4 (1)
4200	18	3 (1)	1	6 (3)
4500	7	0	1	4 (1)

RESPONSES

2 PR'S - (1) NON-SMALL CELL LUNG CANCER
(DISAPPEARANCE OF METASTATIC LYMPH NODE)
(2) METASTATIC SARCOMA
(SHRINKAGE OF PULMONARY METS)

3 MR'S - (1) ENDOMETRIAL CANCER
(SHRINKAGE OF PULMONARY METS)
(2) METASTATIC SARCOMA
(SHRINKAGE OF PULMONARY METS)
(3) RETROPERITONEAL LEIOMYOSARCOMA
(SHRINKAGE OF RETROPERITONEAL MASS)

INFLUENCE OF DIPYRIDAMOLE-PALA ON PLASMA URIDINE

PRE-DIPYRIDAMOLE (10 PTS): 3.49 ± 1.48 uM

9 HOURS POST-DIPYRIDAMOLE (10 PTS): 2.29 ± 0.70 uM

1 WEEK POST-DIPYRIDAMOLE (9 PTS): 2.46 ± 0.61 uM

7 HOURS POST-PALA (9 PTS): 0.87 ± 0.23 uM

SUMMARY

1. COMBINATION CHEMOTHERAPY WITH INTRAVENOUS PALA AND DIPYRIDAMOLE ADMINISTERED AT A DOSE OF 50 MG/M^2 P.O. Q6H CAN BE DELIVERED WITH ACCEPTABLE SYSTEMIC SIDE EFFECTS.

2. DOSE LIMITING TOXICITY WITH THIS REGIMEN IS DIARRHEA AND ABDOMINAL PAIN AT A PALA DOSE OF 3900-4200 MG/M^2 ADMINISTERED EVERY THREE WEEKS.

3. THIS DOSE OF PALA IS 80% THAT DELIVERED SAFELY ON A Q21 DAY SCHEDULE IN PHASE II TRIALS OF THIS AGENT ADMINISTERED ALONE (WITHOUT DIPYRIDAMOLE) SUGGESTING SYNERGISTIC TOXICITY TO THE GASTROINTESTINAL MUCOSA.

4. OBJECTIVE ANTI-TUMOR RESPONSES WERE OBSERVED IN SEVERAL PATIENTS WITH ADVANCED MALIGNANCIES.

CONCLUSION

BECAUSE OF THE UNIQUE MECHANISM OF ACTION OF THIS TWO DRUG COMBINATION AND THE SUGGESTION OF CLINICAL ACTIVITY IN THIS PHASE I TRIAL, FURTHER INVESTIGATION OF THIS TREATMENT PROGRAM APPEARS WARRENTED.

PHASE I WORKING GROUP

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MECHANISM OF ACTION STUDIES WITH FLAVONE ACETIC ACID (NSC 347512)

T.H. Corbett, M. Bissery, G.G. Chabot, J.D. Crissman,
C. Yost and F.A. Valeriote

Wayne State University School of Medicine
Detroit, MI 48201

Flavone Acetic Acid (FAA) is a new antitumor agent with broad activity against transplantable solid tumors of mice but with only scant or no activity against leukemias and lymphomas.

The technique of alkaline elution was used to study FAA induced DNA lesions in subcutaneously growing Glasgow Osteogenic Sarcoma (GOS) in BDF_1 mice. The technique of Kohn was used, with a fluorometric determination of DNA.

Cells were placed on top of a 0.8 um polycarbonate filter, lysed with a pH 10.0 solution, and washed with 10 ml of pH 10.3 tetrasodium EDTA. The DNA was eluted at a constant rate of 0.04 ml/min with pH 12.2 tetrapropylammonium hydroxide containing EDTA.

The elution profiles of the DNA from GOS cells treated with progressively increasing dosages of irradiation are shown in Figure 1. As the extent of DNA breakage increases (and the sizes of the DNA fragments are reduced), the rate of elution of the DNA through the filter becomes more rapid: note the difference between the 100 and 500 rad dosages (Figure 1).

FAA administered IV to mice bearing SC 200-500 mg GOS tumors produced single strand DNA breaks (SSB) in a time dependent fashion (Figure 2).

The gross effect of the SSB was the production of hemorrhagic necrosis within the tumor, which was histologically evident by 24 hrs. There was, however, no evidence of tumor necrosis at 3, 5 and 8 hrs post treatment, establishing that the SSB predated the necrosis event.

The production of the SSB was dependent on the dosage administered (Figure 3). At efficacious dosages (200 and 235 mg/kg IV) extensive SSB were evident. At lower inactive dosages (T/C values >42%), there were no SSB (Figure 3).

Unlike other DNA breakage agents (e.g. VP-16), FAA did not produce DNA-protein cross-links (Figure 4). If these types of cross-links occurred, treatment of the 5 hr FAA (200 mg/kg) sample with Proteinase K (PK) would have ruptured the links and the DNA would have eluted more rapidly (i.e., nearer to the rate of the 500 rad elution curve) (Figure 4).

Unlike alkylating agents, FAA did not produce DNA-DNA interstrand cross-links (Figure 5). If interstrand cross-links occurred, the rate of elution of the DNA from cells treated with both FAA and 500 rads would have been slower than that with 500 rads alone. As can be seen in Figure 5, FAA plus irradiation produced an elution profile similar to irradiation alone (note the 3 hr sample).

FAA in bolus dosages produces only minimal or no toxicity to the bone marrow of mice or dogs. As expected there was minimal or no SSB in the bone marrow cells at dosages that produced extensive breaks in the tumor (Figures 6 and 7). The area under the concentration x time curve (AUC) for bone marrow cells of mice treated IV with 235 mg/kg of FAA was higher than the AUC in the tumor of mice treated IV with 200 mg/kg of FAA. This established that failure to take up the drug was not the cause of the bone marrow insensitivity.

FAA is only minimally active against P388 *in vivo*. As expected, FAA failed to produce SSB in these cells (Figure 8). One could postulate that FAA is metabolized in the responsive tumors to an active form and that bone marrow cells as well as leukemias and lymphomas are unable to efficiently accomplish this activation step.

Supported by grants CA-38137, CA-34144 and the Wayne State University Ben Kasle Trust for Cancer Research.

SUMMARY

Mechanism

1. FAA produced single strand breaks (SSB) in solid tumors in a dose and time dependent fashion.

2. The SSB were not repaired.

3. There was minimal or no DNA-DNA or DNA-protein cross-links.

4. FAA produced a hemorrhagic necrosis that was histologically evident at 24 hrs (but not at 3, 5 and 8 hrs).
   SSB occurred at 3, 5 and 8 hrs.

5. FAA did not produce SSB in bone marrow cells or P388 leukemia.

LEGEND FOR FIGURES:

Fig 1. Irradiation Control: Alkaline elution profiles of DNA from Glasgow Osteogenic Sarcoma (GOS) cells trom 300-600 mg SC tumors treated with progressively increasing dosages of Cs irradiation. All samples were also treated with proteinase-K (PK).

Fig 2. Time Course Study - FAA: Alkaline elution profiles of DNA from 300-600 mg SC GOS tumors treated IV with 200 mg/kg of FAA. The tumors were harvested at various times post treatment (as indicated on the Figure). An irradiated control and an untreated control were included.

Fig 3. Dose - Response Study - FAA: Alkaline elution profiles of DNA from 300-600 mg SC GOS tumors treated IV with various dosages of FAA. The tumors were harvested 8 hr post treatment with FAA. An irradiated control and an untreated control were included.

Fig 4. Lack of FAA-induced DNA-Protein cross-links: Alkaline elution profiles of DNA from 300-600 mg SC GOS tumors treated IV with 200 mg/kg of FAA. The tumors were harvested 3 and 5 hr post teatment. A sample of each was treated with PK to break DNA-protein cross-links (if they occurred). Irradiated controls (plus and minus PK) and an untreated control were included.

Fig 5. Lack of FAA induced DNA-DNA cross-links: Alkaline elution profiles of DNA from 300-600 mg SC GOS tumors treated IV with 200 mg/kg of FAA. Tumors were harvested 0.5, 3, and 8 hr post treatment with FAA. A sample of each was treated with 500 rads of irradiation. An irradiated control and an untreated control were included.

Fig 6. Lack of FAA induced DNA breaks in femoral Bone Marrow cells (BM): Alkaline elution profiles of DNA from BM and 300-600 mg SC GOS tumors treated with 200 mg/kg of FAA. The tumors and the BM were harvested at 5 hr post treatment with FAA. Irradiated controls and untreated controls were included. The area under the concentration X time curve (AUC) for the tumor was 29% higher than the AUC in the BM at this dosage.

Fig 7. Lack of DNA breaks in BM treated with FAA at 235 mg/kg: Alkaline elution profiles of DNA from BM treated IV with 235 mg/kg of FAA. The BM was harvested at various times post treatment with FAA (as indicated on the Figure). The AUC for the BM treated with 235 mg/kg of FAA was higher than the AUC for the tumors treated at 200 mg/kg.

Fig 8. Lack of FAA induced DNA breaks in P388 leukemia: Alkaline elution profiles of DNA from IP growing P388 cells treated IP with 235 mg/kg of FAA. The tumor cells were harvested 24 hr post treatment with FAA. An irradiated control and an untreated control were included.

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Figure 1

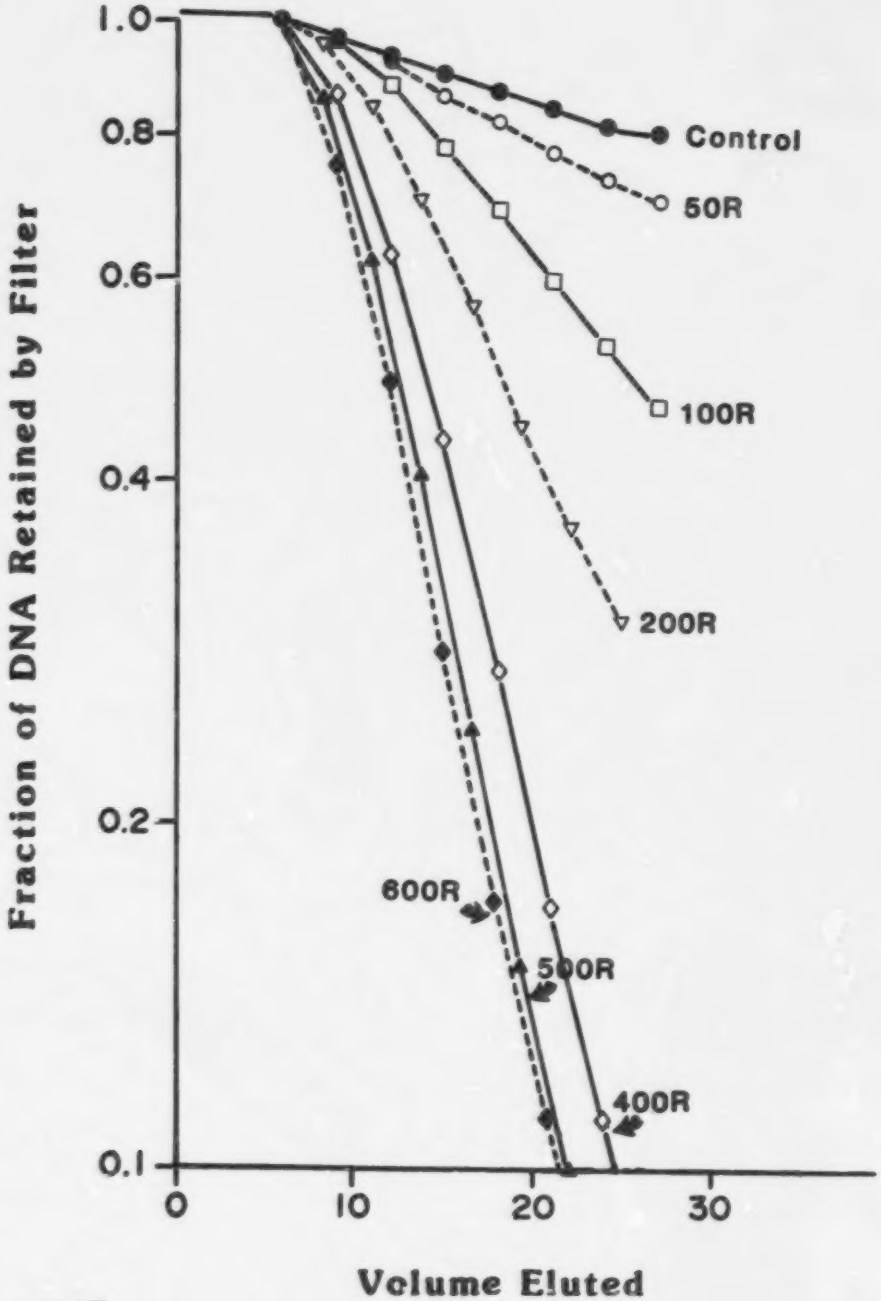

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Figure 2

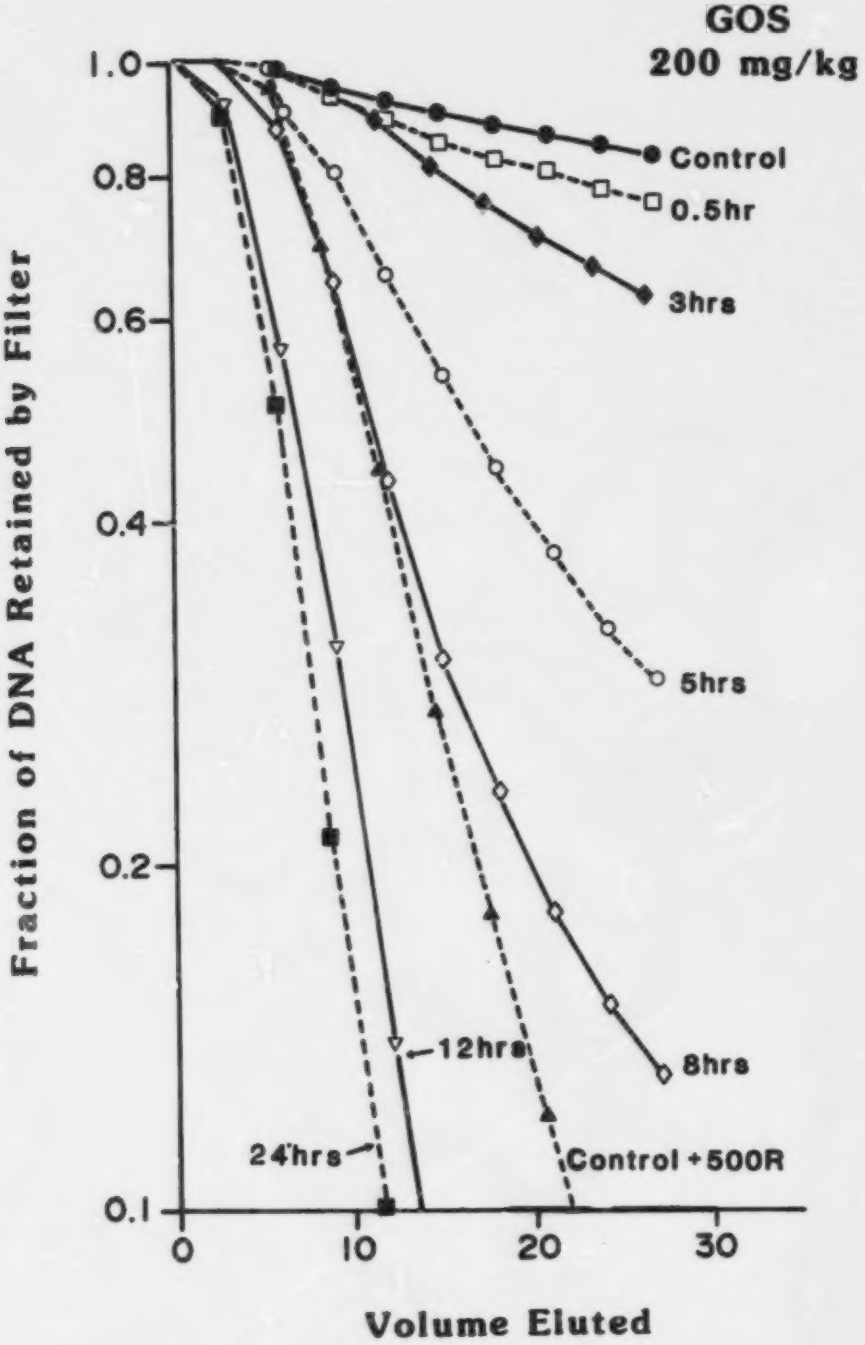

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Figure 3

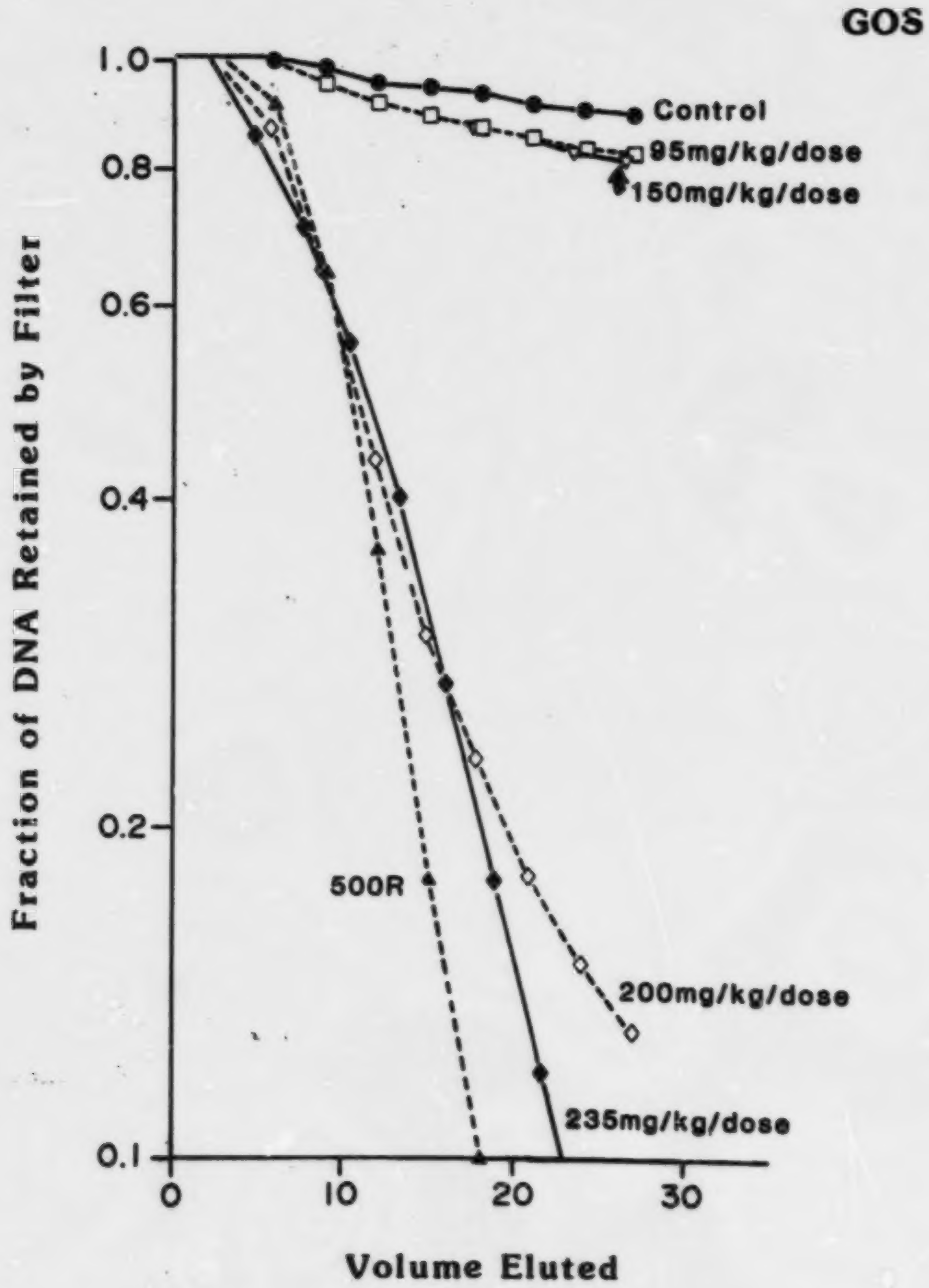

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Figure 4

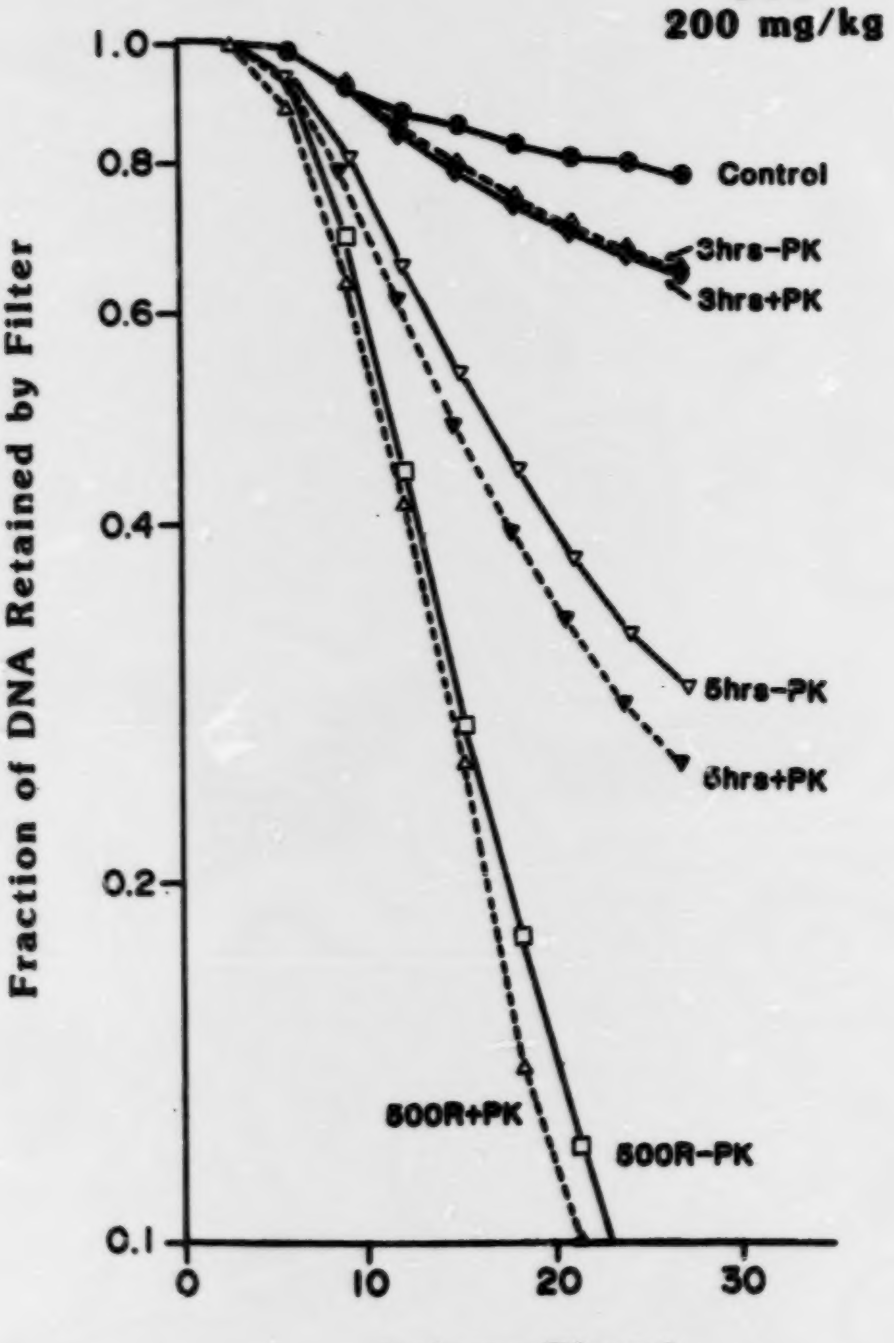

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Figure 5

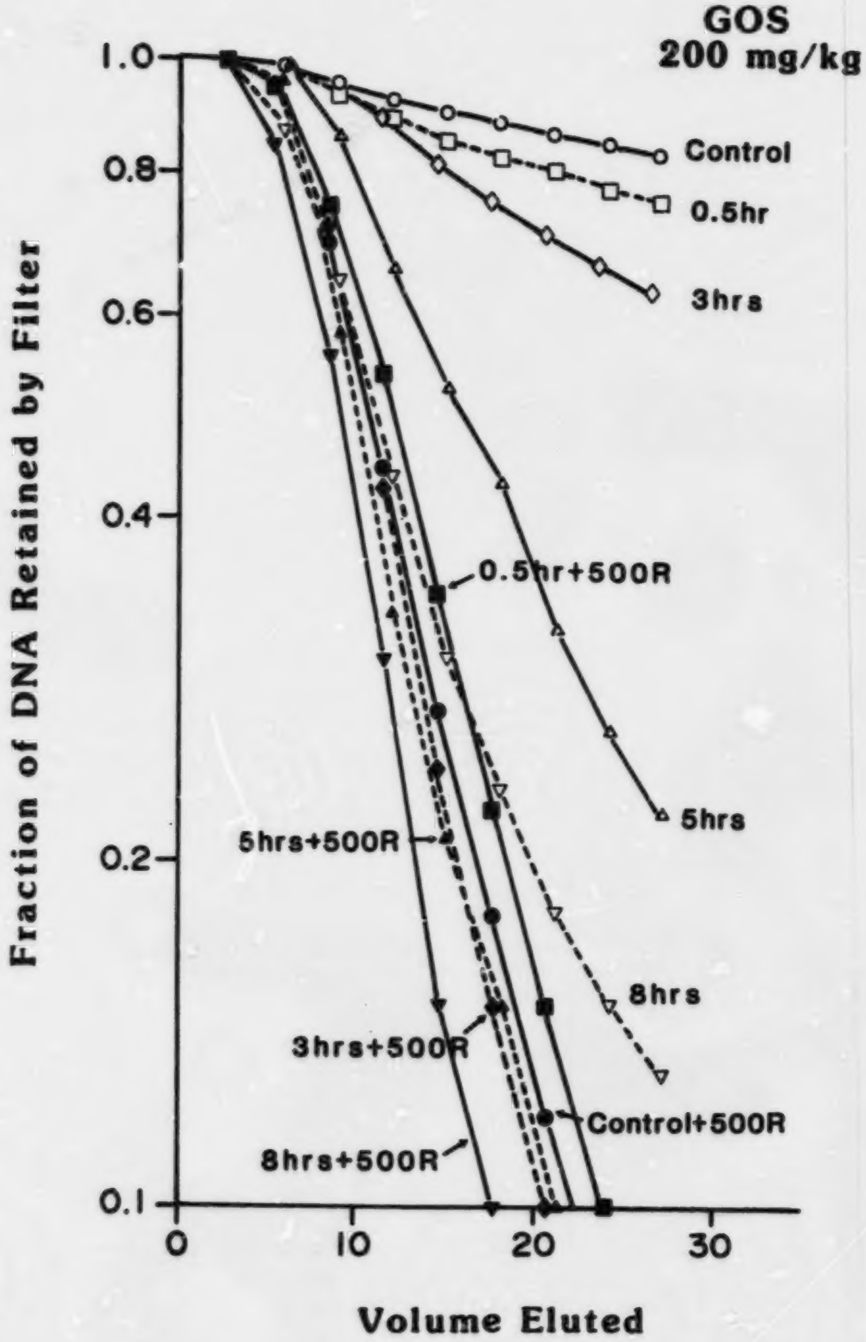

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Figure 6

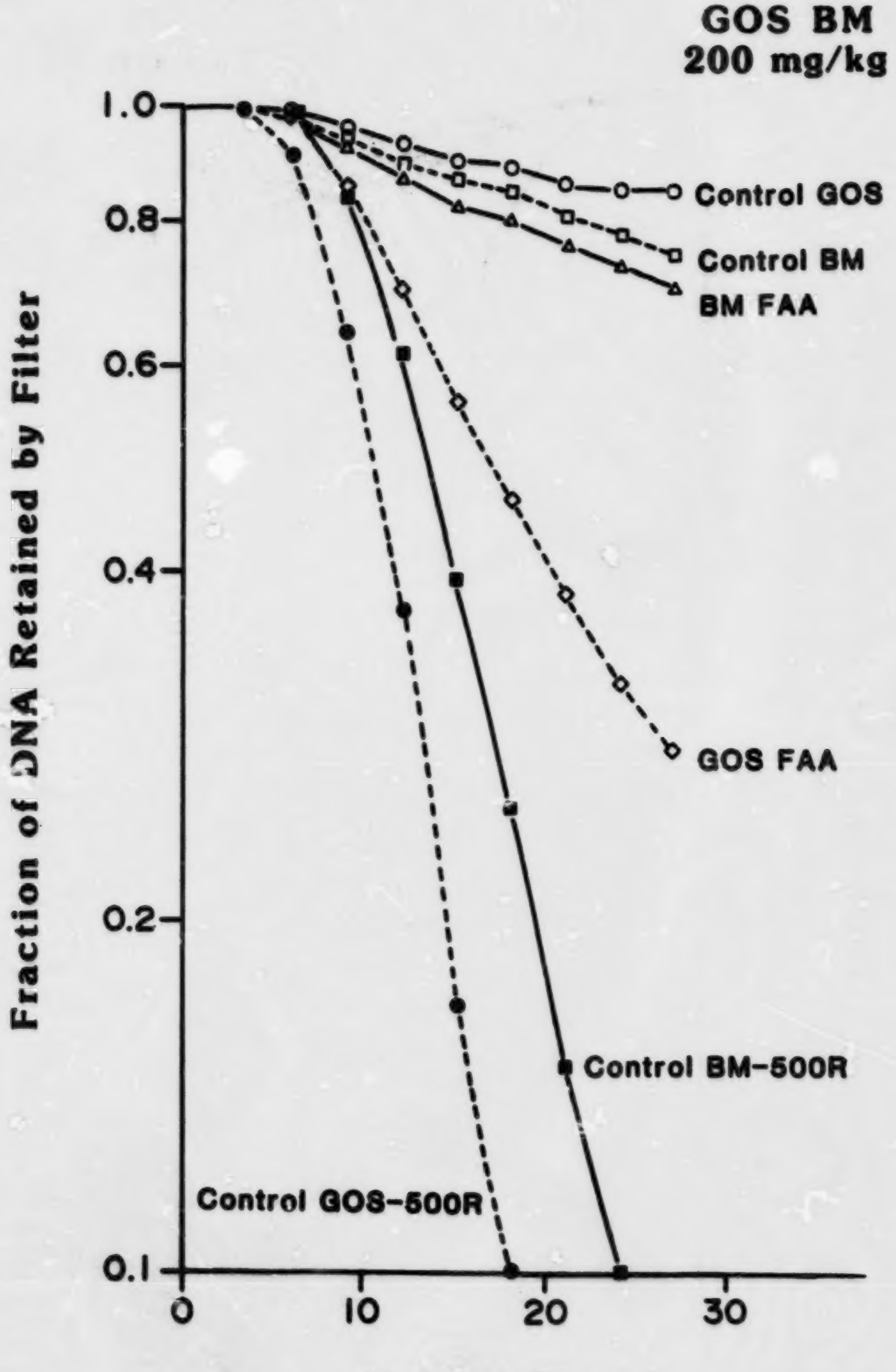

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Figure 7

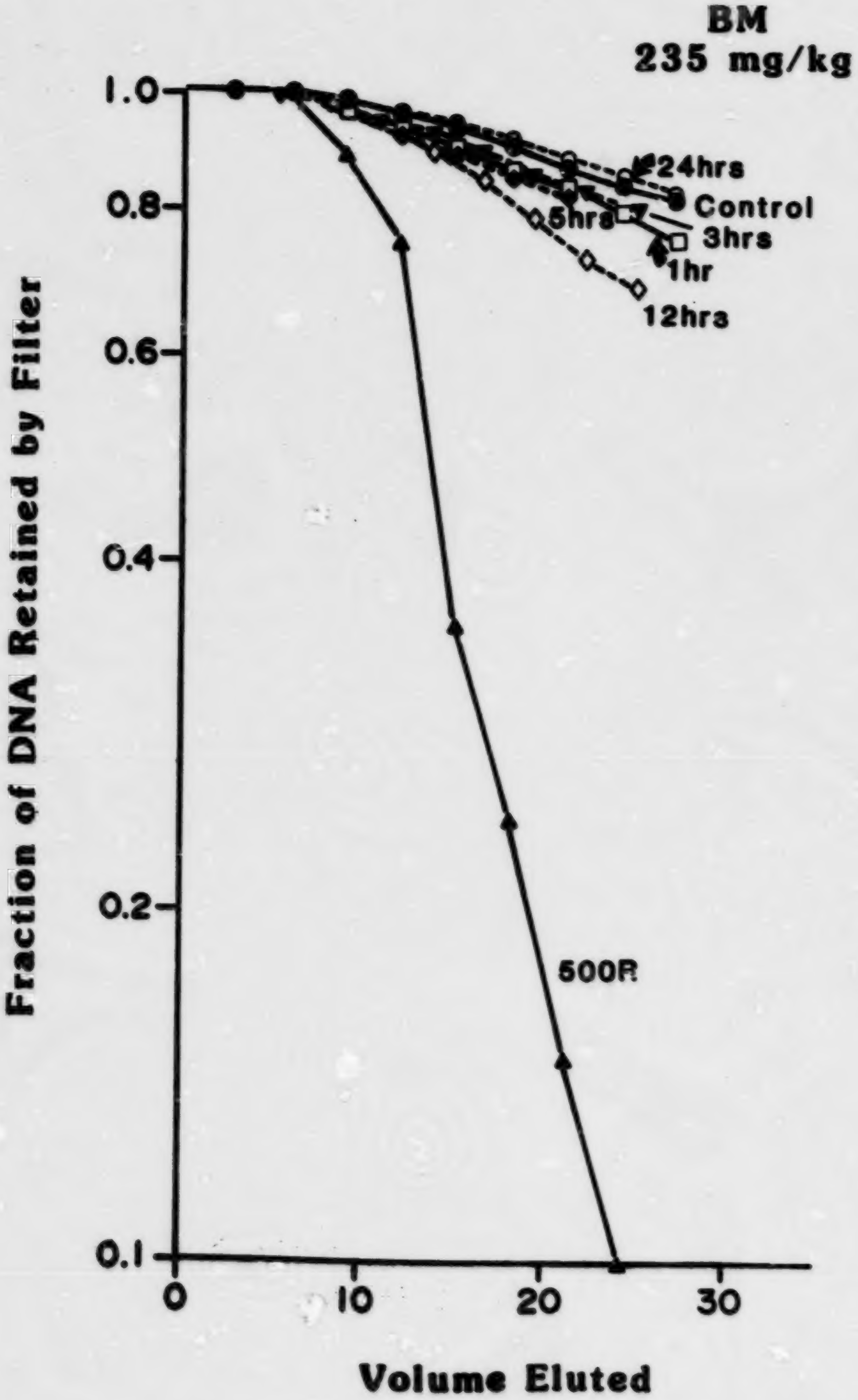

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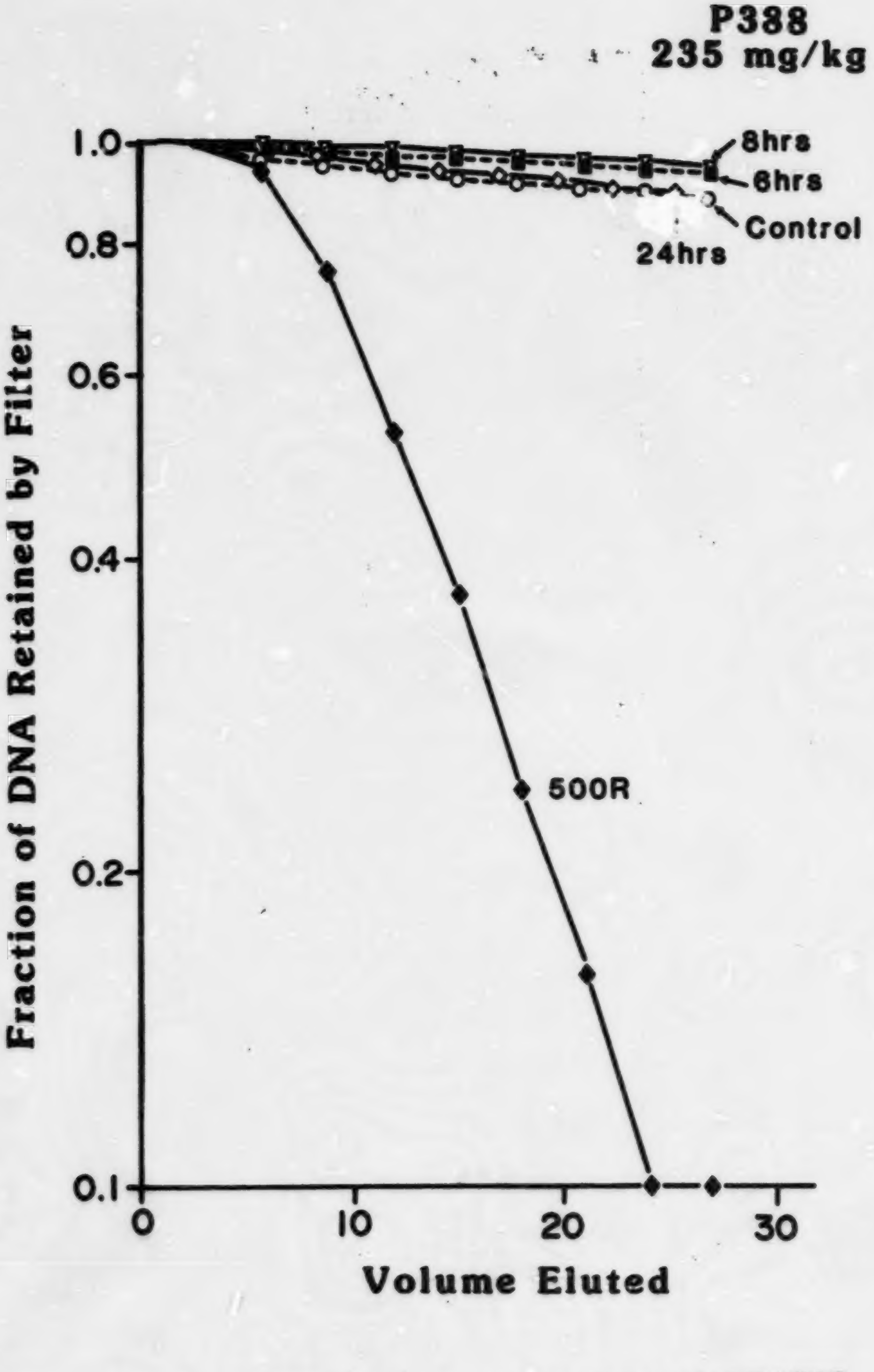
Figure 8



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OSU
July 1986

FLAVONE ACETIC ACID

PATIENT CHARACTERISTICS

NUMBER PATIENTS:	23
MEDIAN AGE:	46 (RANGE 30-79)

INITIAL PERFORMANCE STATUS (PS)

PS	NUMBER OF PATIENTS
0	8
1	8
2	2
3	5
4	0

PRIOR THERAPY

CHEMOTHERAPY:	18
RADIATION THERAPY:	8

FLAVONE ACETIC ACID

PRIMARY SITE

COLORECTAL ADENOCARCINOMA	15
HEAD AND NECK CARCINOMA	3
KIDNEY CARCINOMA	1
LUNG CARCINOMA	1
OVARIAN CARCINOMA	1
MALIGNANT MELANOMA	1
CLOACOGENIC CARCINOMA	1

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FLAVONE ACETIC ACID

DRUG ADMINISTERED

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES
100	3	5
400	5	18
1000	8	19
1500	5	10
2000	4	7
2700	3	4

FLAVONE ACETIC ACID

HEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES	MEDIAN WBC* NADIR	WBC RANGE
100	3	5	5.8	4.9 - 8.4
400	5	18	5.3	4.5 - 12.5
1000	8	19	5.4	3.1 - 11.6
1500	5	10	6.6	3.6 - 13.1
2000	4	7	6.8	4.9 - 10.3
2700	3	4	4.5	3.5 - 14.1

* WBC X $10^3/\mu l$

FLAVONE ACETIC ACID

HEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES	MEDIAN PLATELET NADIR	PLATELET RANGE
100	3	5	221	157 - 771
400	5	18	244	143 - 415
1000	8	19	252	179 - 608
1500	5	10	279	200 - 441
2000	4	7	345	162 - 432
2700	3	4	195	175 - 310

* PLATELET COUNTS X $10^3/\mu l$

FLAVONE ACETIC ACID

NONHEMATOLOGIC TOXICITY

DOSE (MG/M2)	NUMBER COURSES	EPISODES TOXICITY (MEDIAN GRADE)		
		GASTROINTESTINAL	FATIGUE	SEDATION
100	5	1 (2)	-	-
400	18	5 (1)	5 (1)	3 (1)
1000	19	4 (1)	4 (1)	5 (1)
1500	10	1 (1)	3 (1)	-
2000	7	1 (1)	3 (1)	1 (1)
2700	4	2 (1)	1 (1)	1 (1)

FLAVONE ACETIC ACID

HEMATOLOGIC TOXICITY

DOSE LEVEL (MG/M2)	NUMBER PATIENTS	NUMBER COURSES	MEDIAN WBC* NADIR	WBC RANGE	MEDIAN PLATELET NADIR	PLATELET RANGE
100	3	5	5.8	4.9 - 8.4	221	157 - 771
400	5	18	5.3	4.5 - 12.5	244	143 - 415
1000	8	19	5.4	3.1 - 11.6	252	179 - 608
1500	5	10	6.6	3.6 - 13.1	279	200 - 441
2000	4	7	6.8	4.9 - 10.3	345	162 - 432
2700	3	4	4.5	3.5 - 14.1	195	175 - 310

* WBC AND PLATELET COUNTS X $10^3/\mu l$

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FAA
T85-0168
OSU
July 1986

FAA
T85-0168
OSU
July 1986

FLAVONE ACETIC ACID

SUMMARY

SIXTY-THREE COURSES OF FLAVONE ACETIC ACID HAVE BEEN ADMINISTERED TO 23 PATIENTS; NO SIGNIFICANT TOXICITY HAS BEEN OBSERVED. DOSE LEVEL 6 (2700 mg/m2) HAS BEEN COMPLETED AND WE ARE READY TO PROCEED WITH LEVEL 7 (3600 mg/m2). DOSE ESCALATION WILL CONTINUE AS PLANNED UNTIL AN MTD IS ACHIEVED.

UMCC PHASE I - FAA

SCHEDULE:	SINGLE DOSE, Q 3 WEEKS
ADMINISTRATION:	INFUSION IN 500cc NS, IV OVER 1 HOUR
URINE ALKALINIZATION:	SODIUM BICARBONATE (PO +/or IV) TO OBTAIN URINARY PH $\geq$ 7 PRIOR TO AND X 48 HOURS POST FAA INFUSION
ESCALATION SCHEME:	1.0 → 1.5 → 2.0 → 2.7GM/M^2

UMCC PHASE I - FAA

PATIENT CHARACTERISTICS

NO. PATIENTS/NO. COURSES	12/20
FEMALE/MALE	3/9
PERFORMANCE STATUS	
MEDIAN	80
RANGE	(60-100)
AGE	
MEDIAN	65
RANGE	(34-76)
PRIOR THERAPY	
CHEMO	5
CHEMO + RT	4
RADIATION	2
NO PRIOR THERAPY	1

UMCC - PHASE I - FAA

TUMOR TYPES

COLON	5
LUNG (NSC)	5
SARCOMA	1
UNKNOWN 1°	1

T86-0004

UMCC - PHASE I - FAA

NON-HEMATOLOGIC TOXICITY

DOSE GM/M^2	NO.PTS/ NO. COURSES	NAUSEA/ VOMITING	DIARRHEA	"FLUSHED" SENSATION	CNS*
1.0	3/5	0	0	0	1
1.5	3/5	0	2	0	0
2.0	3/5	2	0	0	3
2.7	3/5	0	0	1	3

*CNS EFFECTS: SEDATION - 2, HEADACHE - 1, EUPHORIA - 2,
LIGHTHEADEDNESS - 2,

UMCC PHASE I - FAA

CNS TOXICITY

DOSE GM/M^2	NO.PTS/ NO. COURSES	SEDATION	LIGHT HEADEDNESS	HEADACHES	EUPHORIA
1.0	3/5	1	0	0	0
1.5	3/5	0	0	0	0
2.0	3/5	0	1	1	1
2.7	3/5	1	1	*	1

NO MYELOSUPPRESSIVE EFFECTS

NO ANTITUMOR ACTIVITY OBSERVED

UMCC PHASE I - FAA

FUTURE PLANS

CONTINUE TO ENTER PATIENTS AT HIGHER DOSE LEVELS UNTIL A MTD IS ACHIEVED

T86-0004

FAA ANALYSIS (After Kaye, et al.)

a) 1 ml plasma (diluted to fit the linear portion of standard curve)

b) Add 10 ul of 100 ug/ml dimethylaminobenzaldehyde

c) Add 7 ml chloroform

d) Cap and shake x 30 min

e) Centrifuge 10 min, 12,000 g, 4°C

f) Remove aqueous layer and denatured protein interface

g) Transfer chloroform layer to clean tube and evaporate to dryness under N_2

h) Reconstitute in 200 ul of methanol:water (1:1)

i) Inject 50–150 ul into HPLC

   1) Mobile phase – 5 mM phosphoric acid:methanol:acetonitrile:isopropanol (70:10:10:10) 2 ml/min

   2) Hardware – Alltech C_{18} column (10 um, 25 cm), COPEL ODS guard column, Waters 510 pump, Waters 740 integrator, Perkin-Elmer LC85 UV detector set at 303 nm

FLAVONE ACETIC ACID PHARMACOKINETIC PARAMETERS

Patient	Dosage (gm/m²)	Dose (gm)	(hr) t 1/2α	(hr) t 1/2β	(ug/ml · hr) AUC	(ml/min/m²) CL_{TB}	(l/kg) VD_{ss}
1	1	1.88	0.315	1.39	160	104	0.186
2	1	1.71	0.270	2.45	149.3	112	0.258
3	1	1.43	0.288	2.13	77.4	215	0.338
4	1.5	2.43	0.154	1.29	286.6	87	0.184
5	1.5	3.12	0.520	3.26	366.9	68	0.233
6	1.5	2.73	0.585	7.69	184.1	135	0.437

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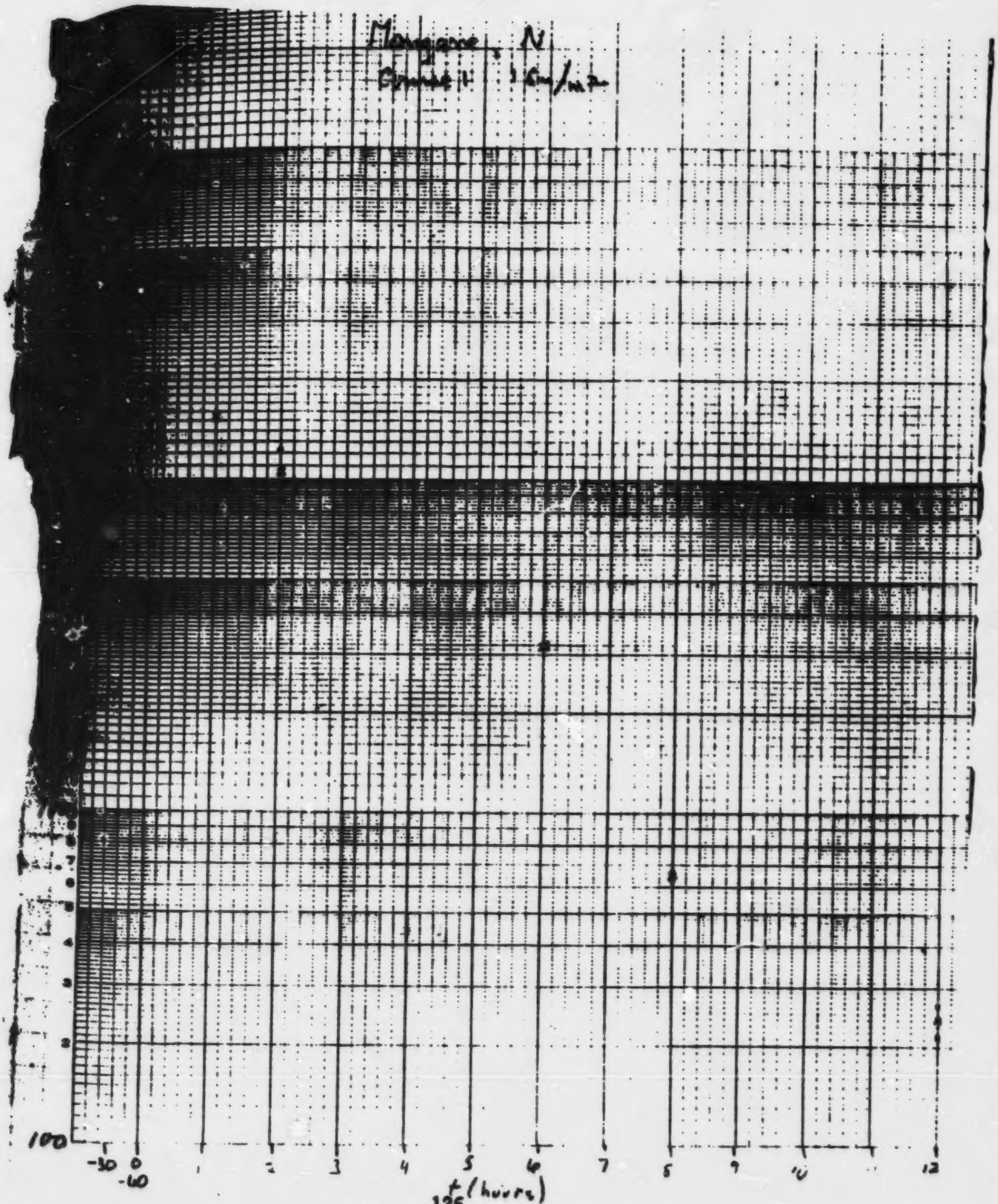
Manganese, N
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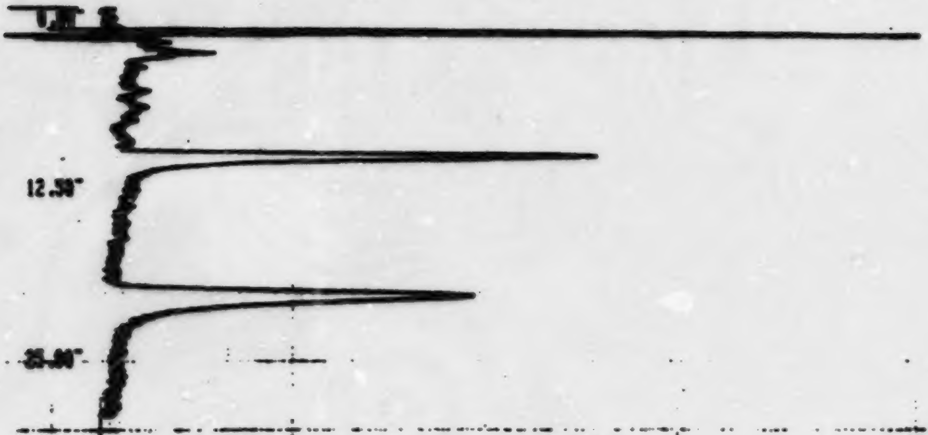
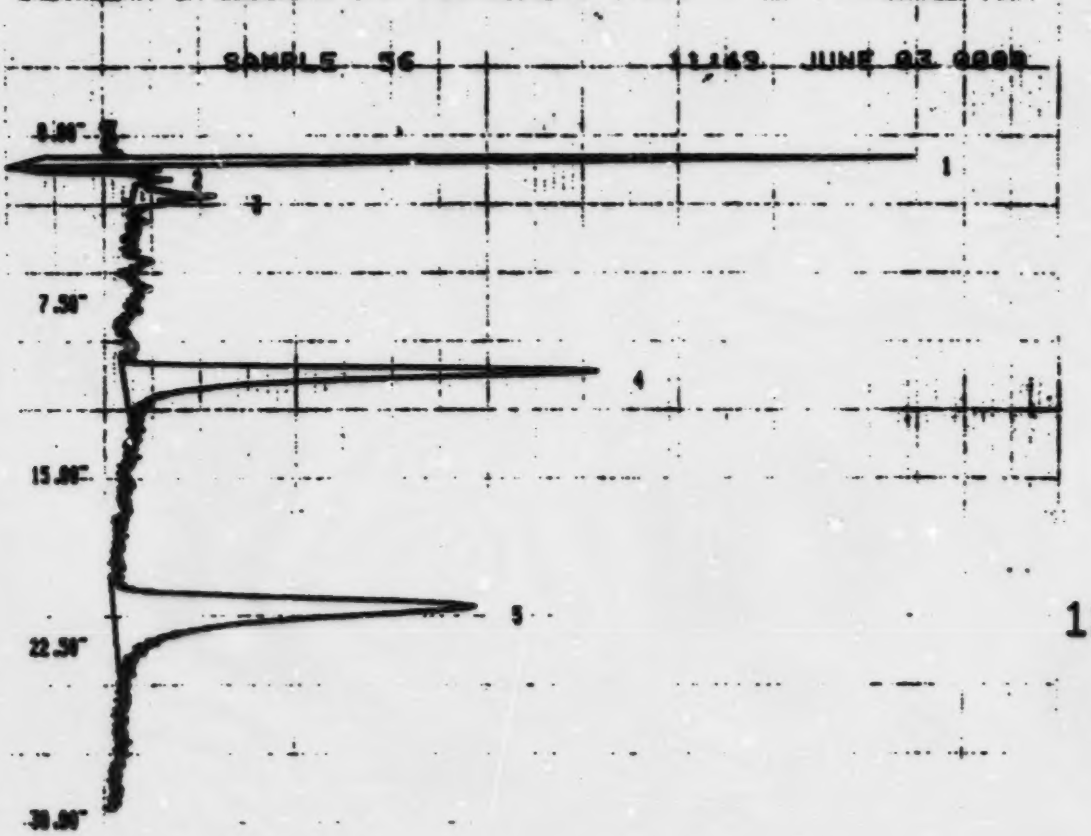
-30
0
-60
1
2
3
4
5
6
7
8
9
10
12
t (hours)

123





124

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In a joint NCI-Walter Reed Medical Center trial, flavone acetic acid has been given as a weekly one hour intravenous infusion. The starting dose was 330 mg/m^2 and four dose escalations have been made to date. The current dose is 2300 mg/m^2. Thirteen patients have been entered in this trial. Toxicity has been minimal, consisting of mild transient somnolence, flushing, and mild diarrhea. There has not been any myelosuppression, alopecia, or hypotension. No objective responses have been achieved to date. The MTD has not been reached and further dose escalations will be made.

FAA SCHEDULE

1. one cycle = 6 weeks
    a. FAA weekly x 4
    b. 2 week rest
2. Off-study criteria - progressive disease after two cycles

FAA ADMINISTRATION

1. i.v. hydration with a liter of D5 1/2 NS over 2 hours urine alkalinisation to pH of 7 or greater
2. FAA is administrated by i.v. infusion over 1 hour

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MODIFIED FIBONACCI SCHEME

330 mg/m^2	3 patients
600 mg/m^2	3 patients
1100 mg/m^2	3 patients
1650 mg/m^2	3 patients
2300 mg/m^2	3 patients
3000 mg/m^2	3 patients
3900 mg/m^2	3 patients

+ 30% previous level

FAA - PHASE I CLINICAL TRIAL

PATIENT CHARACTERISTICS

No. of Pts	
Entered	13
Evaluable	12
Sex, M/F	10/3
Age, median (range)	59 (36-70)
P.S. (Karnofsky), median (range)	80% (70-90)
Prior Treatment	
None	0
Radiotherapy	7
Chemotherapy	13
Chemo and Radiotherapy	7
Median No. of Previous Drugs (range)	2 (1-3)

FAA
T86-0017
NCI
July 1986

TUMOR TYPES

6 colon

4 NSCLC

2 ovary

1 melanoma

TOXICITY

Minimal

nausea - mild and transient

mild diarrhea - 4

hot flushing - 1

weakness, somnolence - 3

No myelosuppression

No alopecia

No hypotension

No objective responses

2 have progressed and died

2 have progessed and are alive

1 has dropped out

8 are stable and remain on study

San Antonio Phase I Trial of Flavone Acetic Acid

Slide. This study schedule was a weekly times four repeated every five weeks The mode of administration was a one hour infusion in 250 cc's of normal saline. To date we have only carried out three dose escalations, 330, 660 and 1100 mg/m^2.

Slide. A total of 6 patients have been entered on study with a median age of 64 and a median performance status of 1. One patients had no prior chemotherapy. The tumor types in patients entered on this study are listed on this slide.

Slide. No toxicities attributable to flavone acetic acid have yet been noted.

Slide. We have one 64 year old male with malignant fibrocistiacytoma who received 330 mg/m^2 per week times four who has had a 25% decrease in lung modules and no change in the primary tumor. This continues at two months after initiation of this agent.

Slide. Our plans are to continue dose escalations with Flavone Acetic Acid.

San Antonio Phase I Trial of Flavone Acetic Acid

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1) Schedule: weekly x 4 repeat every 5 weeks
2) Mode of administration:
   1 hour infusion in 250 cc N.S.
3) Escalation scheme: 330, 660, 1100 mg/m^2

Patient Characteristics

Number of patients	6
Male/Female	5/1
Ages in years	
Median	64
Range	(44-72)
Performance status	
Median	1
Range	(0-2)
No prior chemotherapy	1
Prior chemotherapy	5
Tumor types	
Non small-cell lung	2
Colon	1
Pancreas	1
Sarcoma	1
Breast	1

Toxicities to Date

None attributable to FAA

Responses

Sixty-four yo male with malignant fibrous histiocytoma who received 330 mg/m^2/week x 4 has had 25% decrease in lung metastases (no change in primary tumor). Continues at 2 months.

Plan

Continue dose escalations

FAA
T85-0168
OSU
July 1986

FLAVONE ACETIC ACID

Pharmacology Report

THE OHIO STATE UNIVERSITY

INTRODUCTION

Clinical pharmacokinetic studies have been conducted in parallel with the phase I evaluation of flavone acetic acid (NSC 347512, FAA). The present report describes the plasma pharmacokinetics in 20 patients following a one hour infusion of the drug on the single schedule, the renal excretion in 9 of the patients and the progress to date in characterizing the metabolites of FAA.

METHODS

1. Analytical Method

An HPLC assay was developed for the quantitation of FAA in plasma and urine. Two calibration curves were employed for the assay of patient plasma samples using 1.0 and 0.1 ml of plasma for the low and high concentration ranges, respectively (Table 1). Isolation of FAA from the biological samples after the addition of internal standard (Table 1) was achieved by acidification of the samples and extraction into diethyl ether (Table 2). Following evaporation of the extract to dryness and reconstitution of the residue in the mobile phase, isocratic reversed-phase liquid chromatography on a C_{18} column with UV detection (300 nm) afforded separation of the parent drug from its metabolites (Table 3). Urine samples were assayed by methods similar to those used for plasma (Tables 1-3).

Calibration curves were prepared daily and all samples were assayed in duplicate. The peak area ratios of FAA to the internal standard were plotted against concentration. The best fit straight line was determined by least squares regression analysis using 1/peak area ratio squared as the weighting factor to calculate the slope, x-intercept and correlation coefficient. The concentration of FAA in the unknown samples were calculated using the results of the regression analysis.

2. Clinical Pharmacokinetics

Blood samples were obtained in plastic disposable syringes which contained a drop of heparin, 1000 units/ml. Pre-dose blood was obtained for each pharmacokinetic study just prior to administration of the drug. A minute timer was used in each study which was started at the beginning of the infusion of the FAA dose. The time at the end of the infusion and the time at the beginning and end of each blood withdrawal was recorded. The time used for the plasma pharmacokinetics was the midpoint of the blood collection period. Each blood sample was immediately centrifuged at 1800 x g for 8 minutes. The plasma sample was

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transferred to a separate test tube and stored at -20° until assayed. Plasma samples were obtained during 30 hours following the infusion of doses of 100 and 400 mg/m^2 and during 72 hours following doses of 1000, 1500 and 2000 mg/m^2.

Prior to administration of FAA, 50 ml of urine was obtained. Following drug administration, 10 ml of urine was removed from each void for the first 24 hours and the remainder was pooled. On subsequent days (days 2 and 3), urine voids were pooled to obtain 24 hour urine collections and 50 ml of each 24 hour urine collection was removed. The time, pH and volume of each void was recorded. All urine samples were stored at -20° until assayed.

Both two and three compartment open models utilizing appropriate infusion equations for the intravenous injection period were evaluated for "goodness of fit" to the patient plasma concentration-time data at several dose levels. The best fits were obtained using a three compartment open model and $1/y^2_{obs}$ as a weighting term. Each patient data set was fitted by the non-linear least squares computer program NONLIN. Parameters which were iterated were the microscopic rate constants and V_1. In the plasma profiles, the line drawn through the experimentally determined FAA concentrations is from the computer fit of the three compartment open model to the data.

3. Metabolite Characterization

FAA and its metabolites were isolated on an Amberlite XAD-2 column. After washing the column with water, the compounds were eluted from the column with methanol. TLC on aluminum support coated with silica gel 60 with fluorescent indicator using chloroform-methanol (1:1) showed two major spots with R_f values 0.43 (streaked) and 0.79 (identical with FAA). The methanol solution was concentrated under vacuum at 40° to a light brown solid. The residue dissolved in 0.5 ml of methanol-water (1:1) was applied to 4 preparative TLC silica gel GF plates (20 cm x 20 cm, 1 mm thick). The plates were developed with chloroform-methanol (1:1). On each plate the UV visible band with R_f 0.43 was scraped from the plates and the scrapings were combined. The compounds were eluted from the silica gel scrapings by stirring with methanol for 1 hour and after filtration, the solvent was removed under a stream of nitrogen. The cream colored residue was washed with acetone and dried under vacuum.

Following hydrolysis of the metabolites, spots after TLC were detected using sulfuric acid-ethanol spray and heating. Paper chromatography was performed on Whatman No. 3M paper (1" x 6.5") with either (solvent A) ethyl acetate-methanol-water-acetic acid (65:20:10:7.5, v/v/v/v) or (solvent B) ethyl acetate-methanol-water-formic acid (18:3:4:1, v/v/v/v). The spots on paper were detected when the chromatogram was successively dipped in silver nitrate solution in acetone, aqueous methanolic potassium hydroxide, and aqueous sodium thiosulfate solution.

FAB mass spectra were obtained with a Kratos MS-30 mass spectrometer. UV spectra during chromatography were obtained with a Hewlett-Packard model 1040M diode array detector.

RESULTS AND DISCUSSION

1. Analytical Method

The extraction procedure and chromatography are summarized in Tables 1-3. Employing the analytical mobile phase, chromatograms exhibited single peaks corresponding to the metabolites and the parent drug with retention times of approximately 5 and 12 minutes, respectively (Fig. 1). A three-dimensional chromatogram of an extract of patient plasma obtained at 10 hours after the start of a one hour infusion of 2000 mg/m^2 of FAA demonstates the similarity of the UV spectra of the metabolites to that of FAA (Fig. 2). UV spectra from 230 nm to 400 nm determined at the upslope, apex and downslope of the chromatographic peak due to the parent drug were superimposed indicating that the peak is apparently homogeneous (Fig. 3). The spectra exhibit broad maxima at 255 nm and 300 nm.

Figures 4-6 show typical calibration curves corresponding to the low and high plasma concentration ranges and the urine standards. The mean extraction efficiency of FAA from 1 ml of plasma for concentrations in the range 25-446 ng/ml was 92.99 ± 3.09% and the mean extraction efficiency of the internal standard was 93.23 ± 2.09%.

2. Clinical Pharmacokinetic Studies

The plasma pharmacokinetics are reported for 20 patients treated with 100-2000 mg/m^2 of FAA. Initially, blood samples were obtained at 15, 30 and 45 minutes during the course of the one hour infusion and at frequent intervals to 30 hours after dosing. As dose escalation occurred, it became possible to extend the blood sampling protocol to 72 hours.

Plasma Profiles

Representative FAA plasma concentration-time profiles are shown in Figs. 7-9. The curves drawn through the experimentally determined plasma concentrations represent the computer fit of the data to a three compartment open model. Following one hour infusions of 1000-2000 mg/m^2, the plasma levels generally declined more than 1000-fold during 72 hours.

Apparent Recycling

Evidence of some degree of apparent recycling was observed in the majority of plasma profiles. This effect was seen most clearly in the beta and gamma phases of the profiles (Figs. 10 and 11).

The observed variability in the plasma levels due to apparent recycling, prompted blood sampling every few hours during the terminal phase in order (1) to obtain a reliable terminal phase fit and (2) provide sufficient data for possible evaluation of the apparent recycling.

Several potential explanations of the apparent recycling were evaluated. Urinary pH did not appear to correlate with the recycling behavior; therefore, potential variations in the degree of passive renal tubular reabsorption is thought to be an unlikely explanation. No distinctive pattern was found which correlated recycling peaks or valleys with the time of day. The variation could not be correlated with the time of meals which could trigger gall bladder contractions and possible enterohepatic recycling. Due to the concern that the apparent recycling may have been the result of conversion of a metabolite to the parent drug, blood samples were centrifuged in a refrigerated centrifuge. Refrigerated centrifugation did not eliminate the observed plasma level variability. The apparent recycling remains unexplained. Not all patient studies exhibited the effect.

Mean Pharmacokinetic Parameters

A summary of the mean pharmacokinetic parameters at each dose level for the initial courses is shown in Tables 4-6. The pharmacokinetic parameters show definite dose-dependent behavior. The mean total body plasma clearance decreased from 235.5 to 77.3 ml/min/m^2 as the dose was increased from 100 to 2000 mg/m^2 (Table 4). The coefficient of variation of the mean Cl_T values ranged from 19% to 54% (Table 7). A larger than proportionate increase in the observed peak plasma concentrations was seen as the dose was increased (Figs.7-9).

The harmonic mean half-life corresponding to each of the exponential phases was increased as the dose was escalated (Table 4). With increase in dose, a shift in the mean percentage of the area under the plasma concentration-time curve corresponding to the beta and gamma phases to the alpha phase was observed (Table 5).

The mean steady state volume of distribution, Vd_{ss}, also exhibited dose-dependent behavior, decreasing from 49.2 L/m^2 to 19.3 L/m^2 as the dose was increased from 100 to 2000 mg/m^2 (Table 6). The decrease in Vd_{ss} was the result of decreases in both V_2 and V_3, since the central compartment volume of distribution, V_1, was found to increase with increasing dose.

In contrast to the observed dose-dependent nature of Vd_{ss}, Vd_γ appeared to be dose-independent within the dosing range studied (Table 6). Vd_γ is strongly dependent upon the terminal phase concentrations which in turn are dependent upon the influence of the apparent recycling. While the variation in the terminal plasma concentrations can greatly influence γ, $t_{1/2\gamma}$ and Vd_γ, they have little effect on AUC and Cl_T since the vast majority of the dose has been eliminated by the time the terminal phase becomes dominant. The apparent dose-independent behavior of Vd_γ may be an artifact of the variability in the terminal phase plasma concentrations or, if real, may reflect a relatively small, but high affinity binding tissue located in organs with pseudo-first-order uptake mechanisms. While these tissue binding sites are relatively small in number and contribute little to the Vd_{ss} term, they are able to retain a small fraction of the dose, slowly releasing the drug back into the blood for a prolonged period of time.

The principal influence of the recycling is on γ, the terminal disposition half-life ($t_{1/2\gamma}$), and Vd_{γ}, however, its effect on the total AUC and Cl_T is small. Comparison of NONLIN fits of the 72 hour data with 24 hour truncation of the same data showed only minor differences, amounting to only a few percent, in Cl_T values. Consequently, the important pharmacokinetic parameter for FAA elimination is Cl_T and not the terminal disposition half-life.

Intra-subject Dose Dependency

Dose-dependent pharmacokinetic behavior was also observed within a given patient (Table 8). Patient J.S. was treated with two courses of FAA at dose level 1 (100 mg/m^2) and three courses at dose level 2 (400 mg/m^2). The patient's mean Cl_T at dose level 1 was 232.8 ml/min/m^2 and 168.3 ml/min/m^2 at dose level 2. The patient's respective mean Vd_{ss} values were 62.8 L/m^2 and 28.0 L/m^2. Patient F.I. also exhibited dose-dependent pharmacokinetics upon escalation of the dose. This patient was treated with three courses at dose level 2 (400 mg/m^2) and a single course at dose level 3 (1000 mg/m^2). The patient's mean clearance at the lower dose was 231.3 ml/min/m^2 compared to the higher dose clearance of 117.7 ml/min/m^2. The patient's respective Vd_{ss} values were 40.9 L/m^2 and 14.8 L/m^2.

Parallel First-Order and Saturable Elimination Model

None of the individual plasma concentration-time profiles exhibited classical Michaelis-Menten non-linear curvature. Consequently, the apparent pseudo-first-order exponential decay over the entire dose range must be the result of parallel first-order and saturable Michaelis-Menten elimination pathways (Table 9). To test this hypothesis, mean Cl_T values for each dose level were NONLIN fitted with weights of $1/y^2_{obs}$ as a function of dose (Fig. 12). An excellent NONLIN fit to the equation was obtained which provided the parameters shown in Table 10.

The steady state volume of distribution term, Vd_{ss}, also exhibited a pronounced dose-dependent behavior (Table 6) and was therefore NONLIN fitted to an analogous equation shown in Table 11. The NONLIN fit (Fig. 13) indicated a maximum mean Vd_{ss} of 66.0 L/m^2 and minimum mean Vd_{ss} of 10.6 L/m^2.

The similar dose-dependent behavior of both Cl_T and Vd_{ss} suggests that a common saturable pathway may exist. The rate-limiting step for both drug elimination and drug-tissue binding within an eliminating organ could be a capacity-limited tissue uptake mechanism. One example is the capacity-limited hepatic uptake of organic anions which has been observed for most biliary contrast agents. This capacity-limited hepatic uptake mechanism for organic anions may involve the Y and Z tissue binding proteins. Relatively high concentrations of Y and Z tissue binding proteins are found in both the liver and kidney.

All other dose-dependent pharmacokinetic parameters appear to be secondary factors to the dose-dependent behavior observed in Cl_T and Vd_{ss}.

As dose escalation continues to increase, Cl_T and Vd_{ss} should be approaching their minimum values. The reported minimum Cl_T (42.2 ml/min/m^2) and Vd_{ss} (10.6 L/m^2) are the expected population mean minima. Individual minimum values for each parameter could be expected to be larger or smaller by a factor of 50% or greater.

Due to the similar dose-dependent nature of both Cl_T and Vd_{ss}, the mean residence time, MRT, did not exhibit dose-dependent behavior (Table 4).

The shift in the mean percentage area under the plasma concentration-time curve from the beta and gamma phases to the alpha phase (Table 5) is due to the apparent reduction in the ability of the drug to be carried by the capacity-limited uptake mechanisms of the eliminating and binding tissues. With a larger fraction of the dose forced to remain in the central compartment volume space, the apparent increase in V_1 may reflect a partial saturation of plasma protein binding of FAA (Table 6).

Renal Clearance

The renal clearance, Cl_R, of FAA has thus far been determined in 9 patients treated within the dose range of 1000 to 2000 mg/m^2 (Table 12). The renal clearance values ranged from 17.1 to 79.2 ml/min/m^2 and represent from 25.5% to 61.1% of total body clearance. With the wide inter-subject variability and the narrow dose range for which data is presently available, no conclusions can yet be drawn with respect to the possible dose dependency of renal clearance. However, it is of interest to note that despite some degree of intra-patient variability in both Cl_T and Cl_R, the fraction of renal excretion within a given patient is remarkably uniform (Table 13).

3. Metabolite Characterization

HPLC of patient plasma and urine extracts and the direct injection of urine showed the presence of major metabolite(s). High levels of metabolites were generally seen in chromatograms of the first urine void obtained after dosing. A chromatogram of urine (5 µl) of a patient obtained at 1.25 hours after the start of dosing with 2000 mg/m^2 employing the analytical mobile phase demonstrate the high levels of metabolites excreted (Fig. 14). The FAA peak eluted at approximately 12.4 minutes and the peak at 5.1 minutes corresponds to FAA metabolites. A chromatogram of urine collected 10-11.6 hours after dosing show metabolite levels which exceed that of the parent drug (Fig. 15). Using the analytical mobile phase, the metabolite peak appears to be homogeneous (Figs. 16 and 17) exhibiting a UV spectrum very similar to that of the parent drug (Fig. 18).

In the course of exploring alternate chromatographic systems to establish peak purity, it was found that the metabolite peak was partially separated when the methanol component of the mobile phase was reduced from 42% to 30% (Fig. 19). Under these chromatographic conditions, the parent drug elutes from the column at approximately 50 minutes. Three-dimensional chromatograms of the metabolites eluting at 17.4 and 18.8 minutes (Figs. 20 and 21) show the similarity of the UV spectra with that of the parent drug. A comparison of the UV spectra determined at the apex of the metabolite chromatographic peaks with spectrum of FAA in the sample (Fig. 22) illustrates the similarity of the chromophores of the compounds. The chromatographic peak of FAA appears to be homogeneous (Figs. 23 and 24). Thus, the spectral evidence obtained indicates that the analytical method employed to assay FAA in plasma and urine is specific.

The metabolites were isolated from patient urine by means of an XAD-2 resin column and separated from the parent drug by preparative thin layer chromatography. The R_f value of the material obtained from the preparative plates was 0.43 whereas the FAA showed R_f 0.79. An FAB mass spectrum (Table 14) in glycerol matrix indicated that the metabolite was a glucuronide (M^+ = m/e 456).

The isolated material when treated with 0.1 N sodium hydroxide was rapidly hydrolyzed (1 min) to flavone acetic acid (TLC) (Table 15). The material gave a positive Molish test for carbohydrate and a positive Dische's test for glucuronic acid. TLC (chloroform-methanol, 1:1 or methanol) after alkaline hydrolysis of the metabolite was carried out. The plates when sprayed with ethanolic sulfuric acid and heated showed a black charred spot at the origin and at R_f 0.62. TLC (methanol) of glucuronic acid, which served as the control, showed the identical R_f value. Paper chromatography of the alkaline metabolite hydrolysis mixture with solvents A and B afforded R_f values 0.13 and 0.05, respectively. D-glucuronic acid, which served as a control, exhibited identical R_f values.

The isolated metabolite was hydrolyzed by β-glucuronidase to glucuronic acid and FAA. After incubation of an aqueous solution of the metabolite (1 mg/0.2 ml) with β-glucuronidase (1 mg) in acetate buffer (0.3 ml, pH 6) at 37° for 4 days, TLC (chloroform-methanol, 1:1) showed no unreacted metabolite and the formation of FAA. Paper chromatography with solvent A gave a spot with R_f 0.13, identical to that of D-glucuronic acid, which was used as a control.

PHARMACOKINETIC AND METABOLIC SUMMARY

1. Flavone acetic acid was eliminated by a pseudo-first-order process and the plasma levels exhibited apparent tri-exponential decay within the dose range of 100 to 2000 mg/m^2.

2. Computer fits of the plasma levels to a three compartment open model were obtained using NONLIN.

3. After one hour infusions of 1000-2000 mg/m^2, the plasma levels generally declined more than 1000-fold during 72 hours after dosing.

4. Dose-dependent pharmacokinetics were reflected in a larger than proportionate increase in both the observed peak plasma concentration, $Cp_{max\ obs}$, and the area under the plasma concentration time curve, AUC, as the dose was increased from 100 to 2000 mg/m^2.

5. The dose-dependent pharmacokinetics was demonstrated by a decrease in the mean total body plasma clearance from 235.5 to 77.3 ml/min/m^2 as the dose was increased from 100 to 2000 mg/m^2.

6. With increase in dose (100 to 2000 mg/m^2), the harmonic mean half-lives of each of the three exponential phases of the plasma profile were prolonged: $t_{1/2\alpha}$ from 3.12 min to 44.97 min; $t_{1/2\beta}$ from 0.67 hr to 2.34 hr and $t_{1/2\gamma}$ from 6.98 hr to 18.30 hr.

7. A shift in the mean percentage of the area under the plasma concentration-time curve corresponding to the beta and gamma phases to the alpha phase was observed with an increase in dose (100 to 2000 mg/m^2): alpha phase, from 33.2% to 74.1%; beta phase, from 36.6% to 15.8%; gamma phase, from 30.2% to 10.0%.

8. The mean steady state volume of distribution was observed to decrease with an increase in dose (100 to 2000 mg/m^2): Vd_{ss} from 49.24 L/m^2 to 19.30 L/m^2.

9. The mean central compartment volume of distribution was observed to increase with increasing dose (1000 to 2000 mg/m^2): V_1 from 3.48 L/m^2 to 6.32 L/m^2.

10. The decrease of both Vd_{ss} and Cl_T with increasing dose is consistent with a capacity-limited tissue uptake mechanism. Capacity-limited tissue uptake is reflected in a shift in the areas corresponding to the beta and gamma phases of the AUC to the alpha phase.

11. The total body volume of distribution, Vd_γ, was apparently not dependent upon dose for the range of doses studied. This suggests that there is a small tissue binding compartment with extremely tight binding. The reduction in Cl_T with increasing dose and the apparent independence of Vd_γ with dose results in a prolongation of the terminal half-life with increasing dose.

12. Intra-subject dose-dependent pharmacokinetics of FAA was observed.

The mean Cl_T for a patient treated with two courses of 100 mg/m^2 and subsequently with three courses of 400 mg/m^2 was 232.8 $ml/min/m^2$ and 168.3 $ml/min/m^2$, respectively. The respective mean Vd_{ss} values were 62.8 L/m^2 and 28.0 L/m^2.

For another patient treated with three courses of 400 mg/m^2 and one course of 1000 mg/m^2, the mean Cl_T at the lower dose was 231.3 $ml/min/m^2$ and at the higher dose Cl_T was 117.7 $ml/min/m^2$. The respective Vd_{ss} values were 40.9 L/m^2 and 14.8 L/m^2 .

13. Variation in the plasma levels of FAA particularly in the beta and gamma phases of the plasma profiles, was suggestive of a recycling phenomena.

The variation was not apparently due to passive tubular reabsorption resulting from changes in urinary pH. The variation could not be correlated with the time of meals which would result in gall bladder contraction and enterhepatic recycling.

14. The renal clearance, Cl_R, of FAA has thus far been determined in 9 patients treated with 1000 to 2000 mg/m^2 doses. The Cl_R values ranged from 17.1 to 79.2 $ml/min/m^2$. For a given patient, the fraction of the dose excreted in the urine was consistent from course to course.

15. Both Cl_T and Vd_{ss} were NONLIN fitted as a function of dose employing Michaelis-Menten equations with parallel first-order components. Projection from these fits would predict that the mean Cl_T will approach a value of 42.17 $ml/min/m^2$ and the mean Vd_{ss} will approach a value of 10.57 L/m^2 as dose escalation continues.

16. Two major metabolites were observed in chromatograms of patient plasma and urine which are conjugates of the parent drug. Evidence has been obtained that both metabolites are glucuronic acid conjugates.

TABLE 1

VOLUMES OF PLASMA AND URINE TAKEN FOR ASSAY OF FLAVONE ACETIC ACID (FAA)

Low Conc. Curve:	0.01 - 0.50 µg/ml
High Conc. Curve:	0.25 - 7.4 µg/ml
Plasma (Low Conc. Curve):	1.0 ml
Plasma (High Conc. Curve):	2-100 µl
Drug-free Plasma:	0-98 µl
Urine:	100 µl or aliquot diluted with pre-dose urine to 100 µl
Internal Standard:	α-Naphthaleneacetic acid in H_2O
Plasma (Low Conc.)	1 ml (2 µg/ml)
Plasma (High Conc.)	100 µl (20 µg/ml)
Urine	100 µl (20 µg/ml)

TABLE 2

EXTRACTION OF FLAVONE ACETIC ACID (FAA) FROM PLASMA AND URINE

Acidification:	HCl (6N)
Plasma (Low Conc.)	100 µl
Plasma (High Conc.)	10 µl
Urine	10 µl
Extraction Solvent:	Ether, 6 ml
Extraction:	Reciprocating shaker, 10 min
Centrifugation:	1800 x g, 10 min
Evaporation:	To dryness under N_2
Reconstitution:	Mobile phase, 200 µl
Volume Injected:	25-50 µl

TABLE 3

HPLC ANALYSIS OF FLAVONE ACETIC ACID

L.C.:	Isocratic
Column:	Ultrasphere ODS, 5 µm (4.6 mm x 15 cm)
Pre-column:	Guard-Pak, C_{18}
Mobile Phase:	H_2O-MeOH-Acetic acid (56:42:2, v/v/v) containing 0.001 M Sodium Dodecyl Sulfate
Flow Rate:	1.0 ml/min
Detector:	300 nm, 0.010 AUFS
Lowest Conc. on Std. Curve:	10 ng/ml

TABLE 4

FLAVONE ACETIC ACID HARMONIC MEAN HALF-LIVES, MEAN RESIDENCE TIMES AND TOTAL BODY CLEARANCE IN PATIENTS FOLLOWING ONE HOUR IV INFUSION

Dose (mg/m^2)	Patients (n)	$t_{\frac{1}{2}\alpha}$ (min)	$t_{\frac{1}{2}\beta}$ (hr)	$t_{\frac{1}{2}\gamma}$ (hr)	MRT (hr)	Cl_T ($ml/min/m^2$)
100	3	3.12	0.67	6.98	3.19	236.47
400	4	5.25	0.82	8.96	3.73	149.27
1000	6	18.88	0.82	13.65	2.82	107.48
1500	3	12.96	1.41	22.56	2.53	80.92
2000	4	44.97	2.34	18.30	4.20	77.25

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TABLE 5

MEAN PERCENTAGE OF THE AREA UNDER THE FLAVONE ACETIC ACID PLASMA CONCENTRATION-TIME CURVE CORRESPONDING TO EACH OF THE TRI-EXPONENTIAL PHASES AT EACH DOSE LEVEL FOLLOWING ONE HOUR INFUSION

Dose (mg/m^2)	Patients (n)	Alpha Phase %	Beta Phase %	Gamma Phase %
100	3	33.15	36.62	30.22
400	4	31.48	42.74	25.79
1000	6	55.23	36.82	7.97
1500	3	63.04	29.35	7.61
2000	4	74.13	15.84	10.03

TABLE 6

FLAVONE ACETIC ACID MEAN VOLUME PHARMACOKINETIC PARAMETERS

Dose (mg/m^2)	Patients (n)	V_1 (L/m^2)	V_2 (L/m^2)	V_3 (L/m^2)	Vd_{ss} (L/m^2)	Vd_γ (L/m^2)
100	3	3.48	7.05	38.71	49.24	160.18
400	4	3.68	5.24	26.32	35.24	122.44
1000	6	4.84	4.60	11.28	20.71	163.59
1500	3	4.17	2.59	10.06	16.81	158.36
2000	4	6.32	1.75	11.23	19.30	127.66

TABLE 7

VARIATION OF TOTAL BODY CLEARANCE OF FAA IN PATIENTS FOLLOWING ONE HOUR IV INFUSION

Dose (mg/m^2)	Patients (n)	Mean Cl_T ($ml/min/m^2$)	S.D. ($ml/min/m^2$)
100	3	235.3	126.5
400	4	149.3	61.4
1000	6	107.5	20.8
1500	3	80.9	23.1
2000	4	77.3	35.0

TABLE 8

Cl_T and Vd_{ss} OF FAA FOLLOWING MULTIPLE COURSES

Patient	Course	Dose (mg/m^2)	Cl_T ($ml/min/m^2$)	Vd_{ss} (L/m^2)
J.S.	1	100	221.9	76.3
	2	100	243.6	49.2
	3	400	142.6	22.7
	4	400	173.5	32.1
	5	400	188.7	29.1
F.I.	1	400	227.7	38.0
	2	400	263.2	45.6
	3	400	202.9	39.0
	4	1000	117.7	14.8

TABLE 9

PARALLEL FIRST-ORDER AND SATURABLE MICHAELIS-MENTEN ELIMINATION PATHWAYS

$$Cl_T = \frac{V_{max}'}{Km' + Dose} + Cl'$$

V'_{max} = Sum of all maximum velocity terms

Km' = Michaelis constant term

Cl' = Sum of all first-order elimination processes

TABLE 10

NONLIN FIT OF TOTAL BODY CLEARANCE vs DOSE

$V_{max}' = 77{,}421$ mg·ml/min/m^4

$Km' = 302$ mg/m^2

$Cl' = 42.2$ ml/min/m^2

$$Cl_T^{max} = \frac{V_{max}'}{Km'} + Cl' = 297.8 \text{ ml/min/m}^2$$

TABLE 11

DOSE-DEPENDENT BEHAVIOR OF THE STEADY STATE VOLUME OF DISTRIBUTION

$$Vd_{ss} = \frac{V_{max}''}{Km'' + Dose} + Vd_{ss}''$$

$Vd_{ss}^{max} = 66.04$ L/m^2

$Vd_{ss}^{min} = 10.57$ L/m^2

TABLE 12

FLAVONE ACETIC ACID RENAL CLEARANCE (N = 9)

	Range
Doses (14 courses)	1000 - 2000 mg/m^2
Cl_T	54.3 - 136.9 ml/min/m^2
Cl_R	17.1 - 79.2 ml/min/m^2
$\mathrm{Cl}_R/\mathrm{Cl}_T$	25.5 - 60.5 %

TABLE 13

FLAVONE ACETIC ACID RENAL CLEARANCE IN PATIENTS TREATED WITH TWO OR THREE COURSES

Course	Patient	Dose (mg/m^2)	Cl_T (ml/min/m^2)	Cl_R (ml/min/m^2)	$\mathrm{Cl}_R/\mathrm{Cl}_T$ (%)
1	J.B.	1000	131.07	79.24	60.45
2	J.B.	1000	102.17	62.38	61.06
1	G.K.	1000	96.16	32.31	33.61
2	G.K.	1000	88.43	30.27	34.23
3	G.K.	1500	76.78	24.03	31.30
2	D.S.	1000	136.93	74.06	54.09
3	D.S.	1000	144.44	68.55	47.46
1	S.S.	1500	54.32	24.88	45.81
2	S.S.	1500	63.15	30.80	48.78

TABLE 14

FAB MASS SPECTRA OF FAA GLUCURONIDES
(M^+ = m/e 456)

MATRIX	m/e	ASSIGNMENT
GLYCEROL	457	M + 1
	479	M + Na
	587	M + 4Na + K
ERYTHRITOL-THREOTOL (3:1)	457	M + 1
	479	M + Na

TABLE 15

FAA GLUCURONIDES ISOLATED FROM PATIENT URINE

1. HYDROLYSIS WITH 0.1 N NaOH TO FAA [TLC ($CHCl_3$ - MeOH, 1:1) R_f 0.79]
2. HYDROLYSIS WITH β-GLUCURONIDASE TO FAA [TLC]
3. MOLISCH TEST FOR CARBOHYDRATE AFTER ALKALINE HYDROLYSIS
4. DISCHE TEST FOR GLUCURONIC ACID AFTER ALKALINE HYDROLYSIS
5.

CHROMATOGRAPHY	SOLVENT	HYDROLYSIS MIXTURE	D-GLUCURONIC ACID
TLC	MeOH	R_f 0.62	R_f 0.62
PAPER	SOLVENT A	R_f 0.13	R_f 0.13
	SOLVENT B	R_f 0.05	R_f 0.05

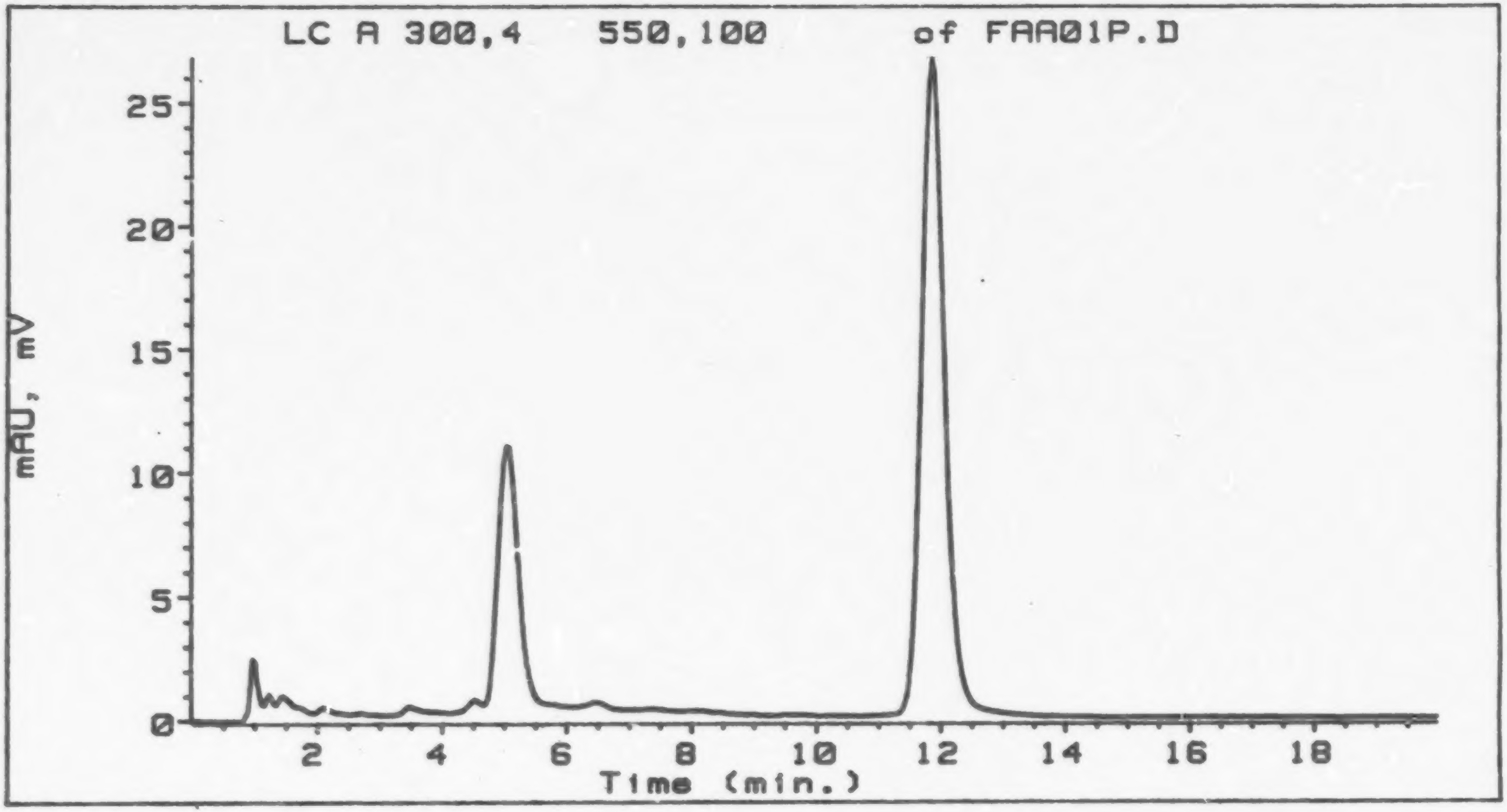

Fig. 1 Chromatogram of an extract of patient plasma obtained at 10 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector at 300 nm. Chromatographic peaks: (5.2 min) metabolites, (11.9 min) FAA.

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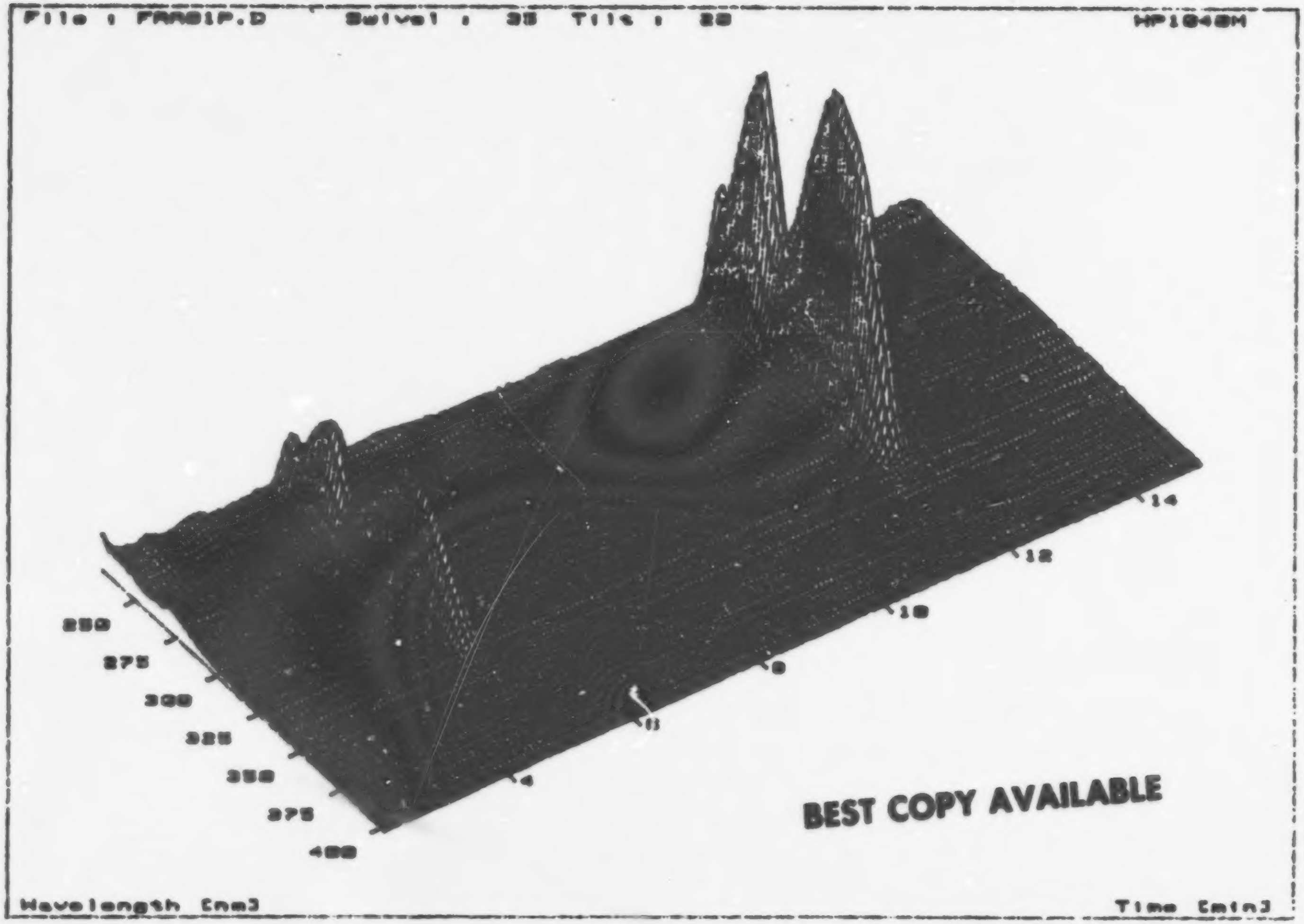

Fig. 2 Three-dimensional chromatogram of an extract of patient plasma obtained at 10 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector. Chromatographic peaks: (5.2 min) metabolites, (11.9 min) FAA.

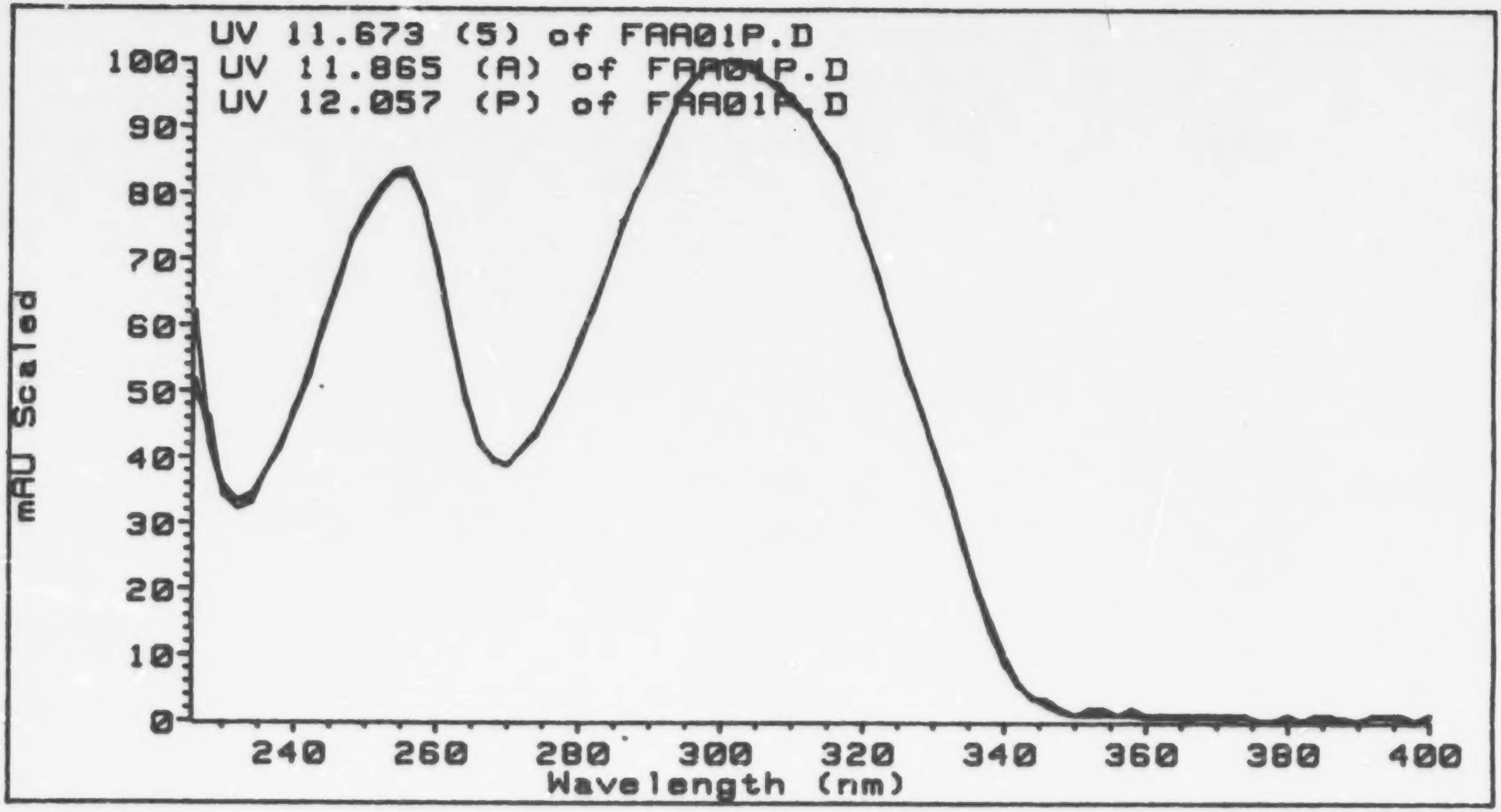

Fig. 3 UV spectra determined at the upslope, apex and downslope of the FAA peak in a chromatogram of an extract of patient plasma obtained at 10 hr after the start of a one hour infusion of 2000 mg/m^2.

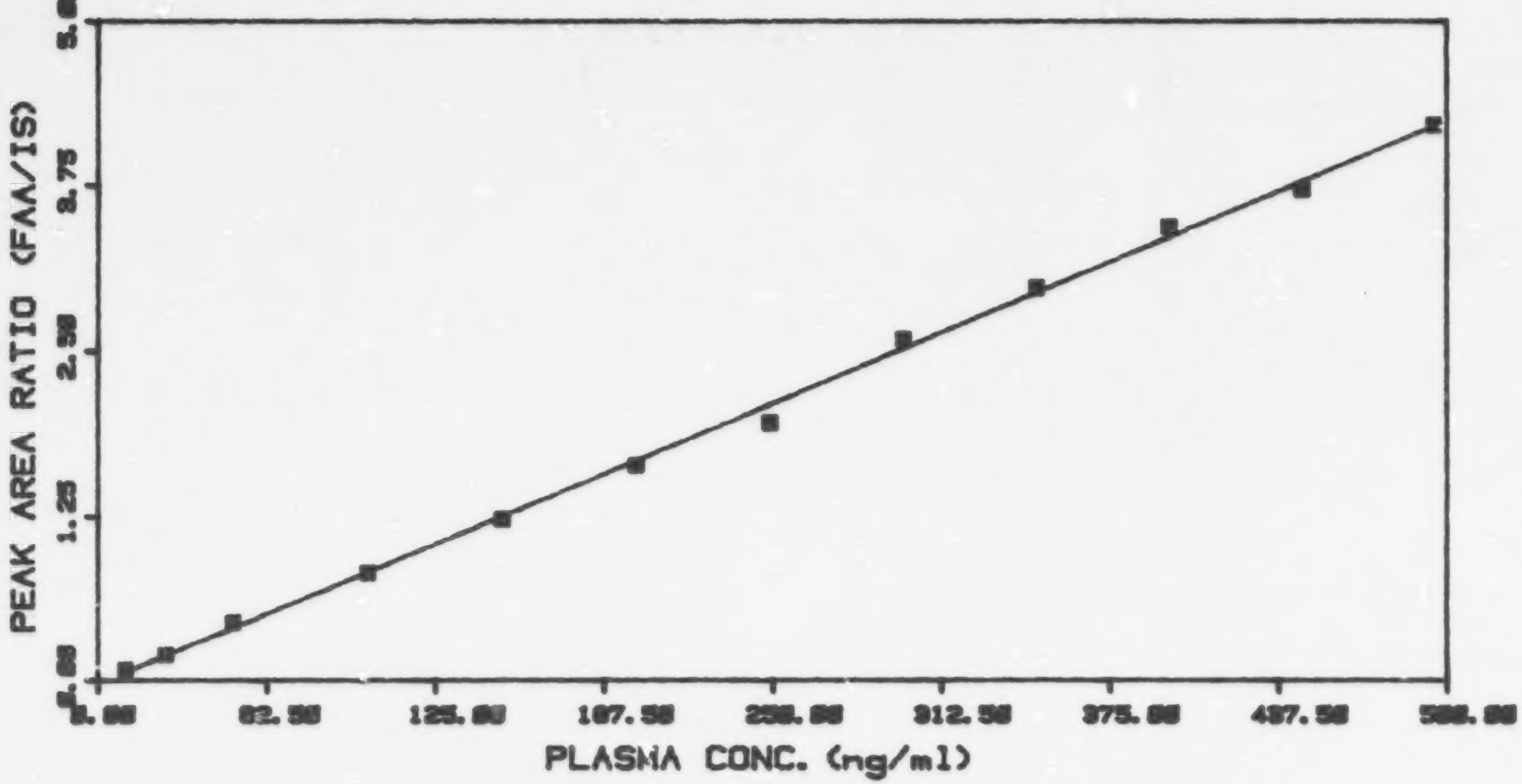

Fig. 4 Calibration curve for flavone acetic acid in patient plasma (low concentration curve). Slope = 0.008415; intercept = -0.003204, r = 0.9992.

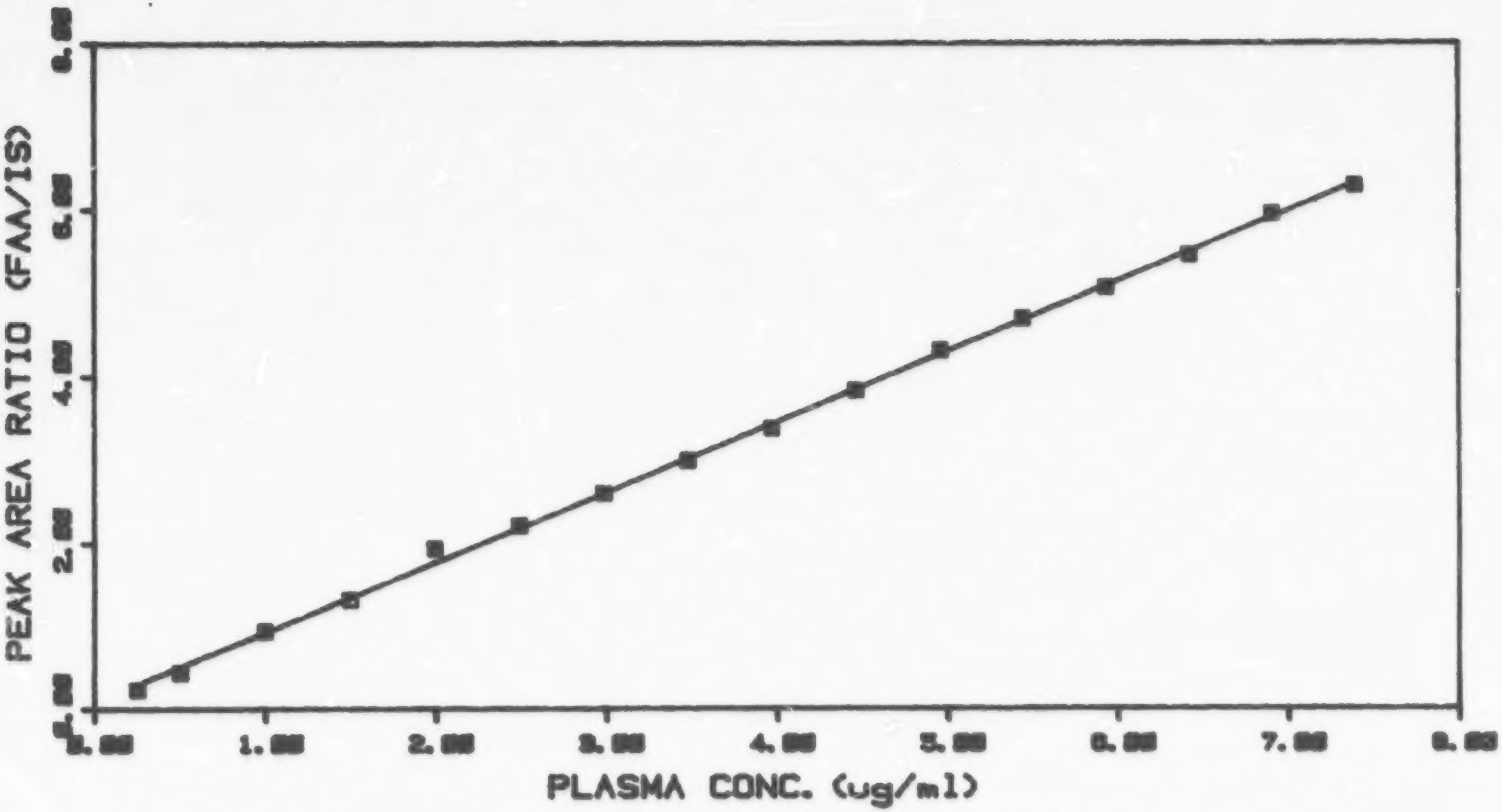

Fig. 5 Calibration curve for flavone acetic acid in patient plasma (high concentration curve). Slope = 0.8947, intercept = -0.002429, r = 0.9998.

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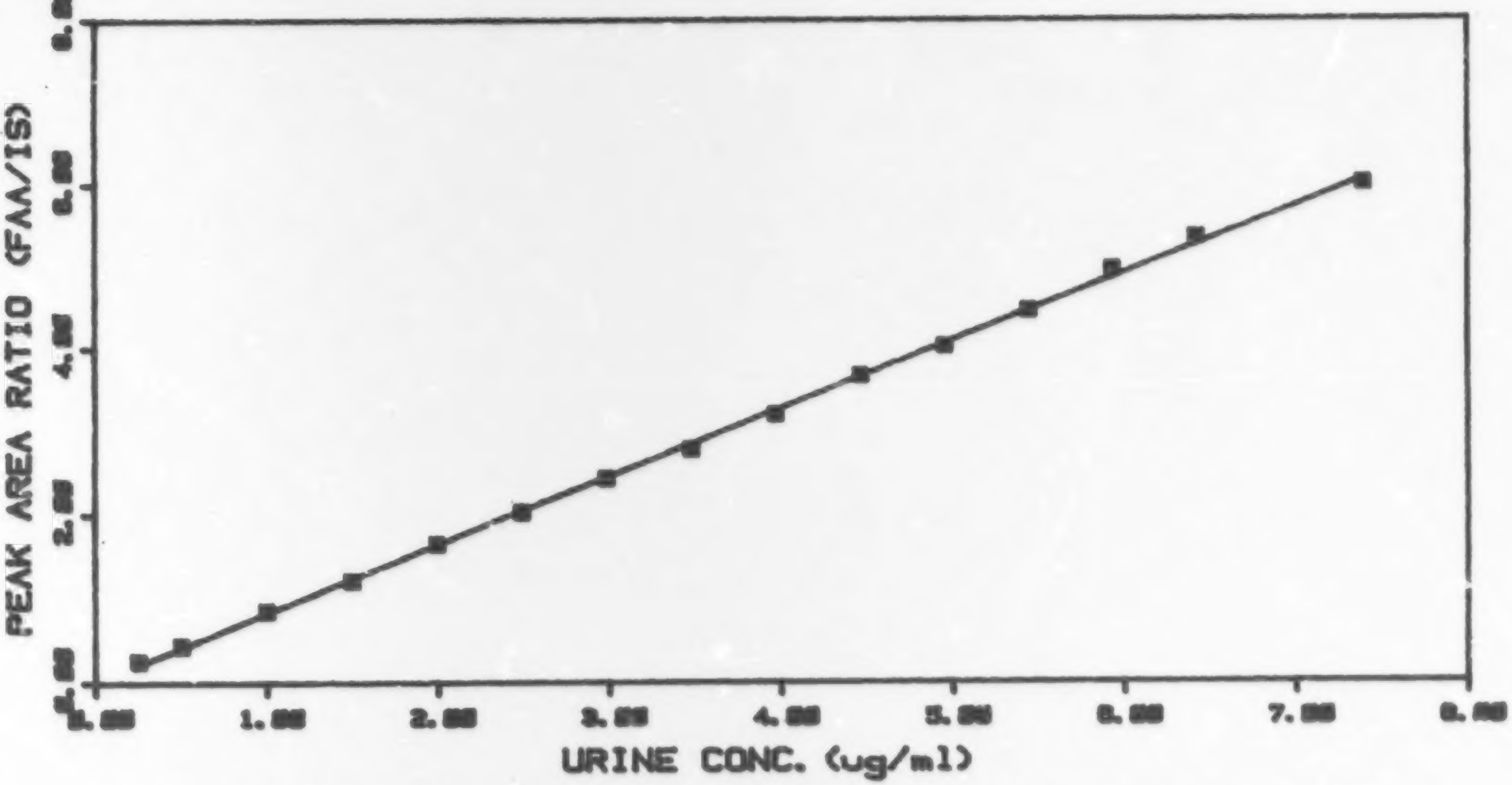

Fig. 6 Calibration curve for flavone acetic acid in patient urine. Slope = 0.7996, intercept = 0.05550, r = 0.9997.

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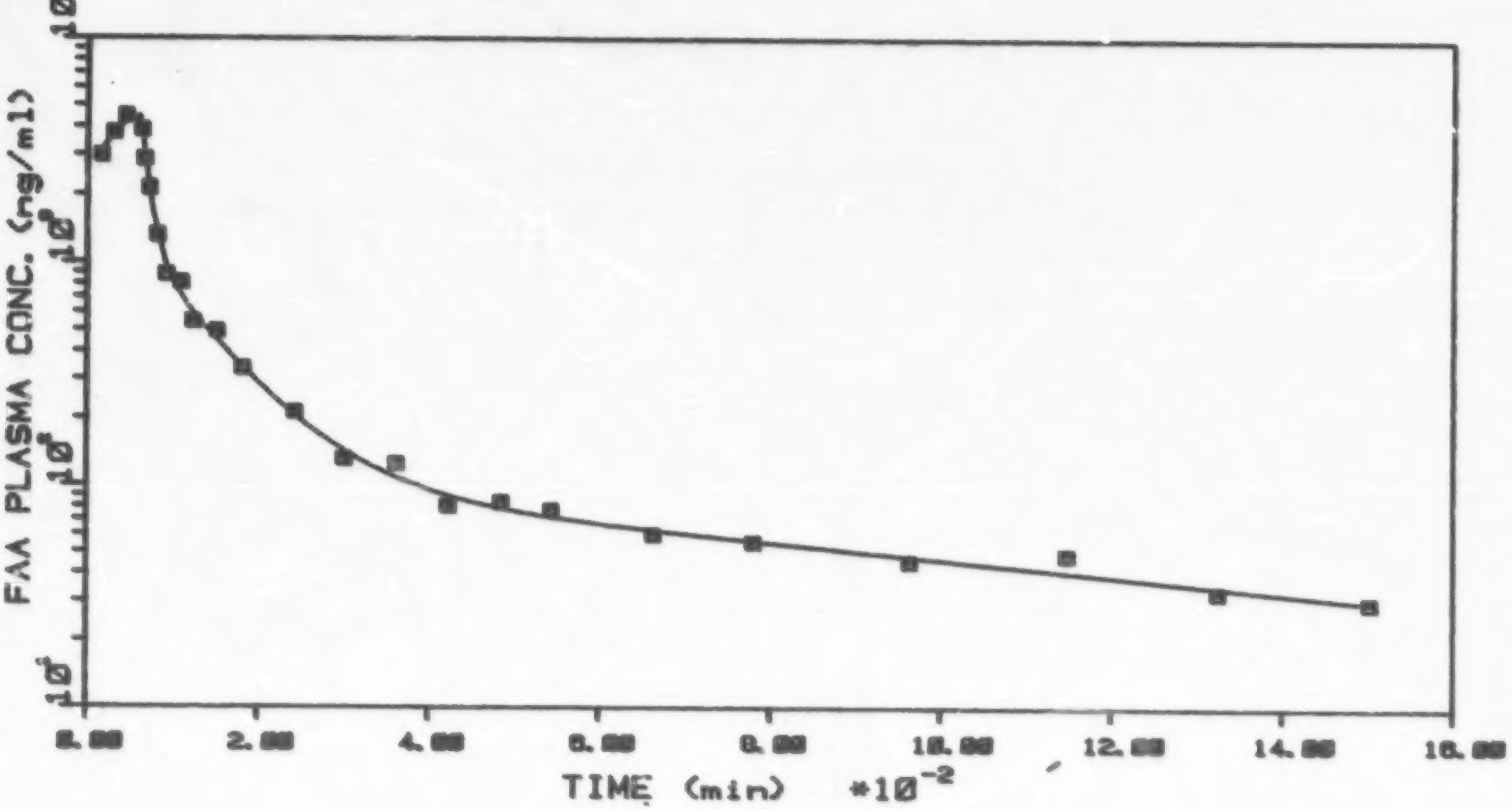

PATIENT J.S.
DOSE = 100 MG/METER SQ. FAA
CLEARANCE = 221.88 ML/MIN/M2

Fig. 7

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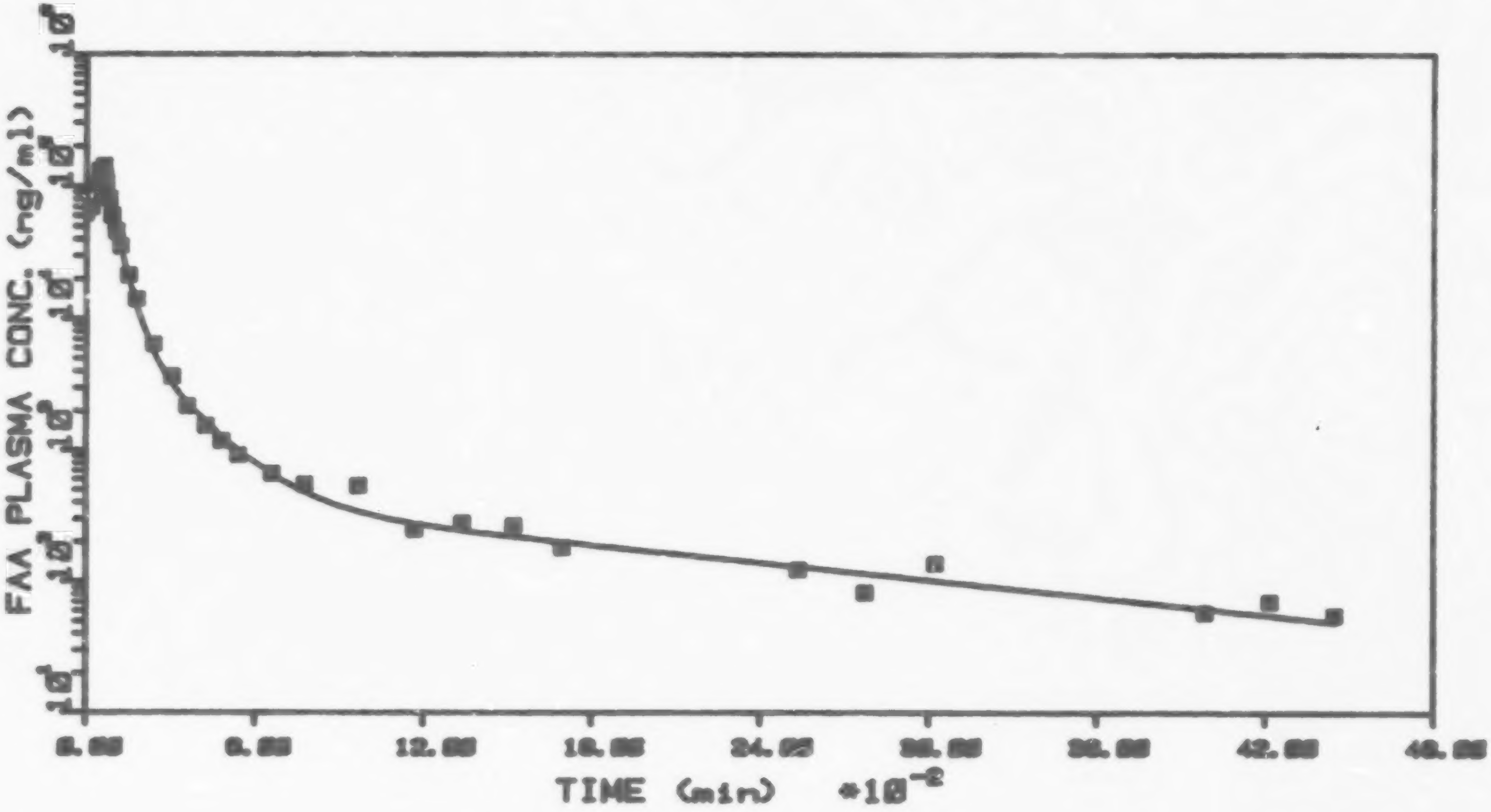

PATIENT M.F.
DOSE = 1000 MG/METER SQ. FAA
CLEARANCE = 76.23 ML/MIN/M2

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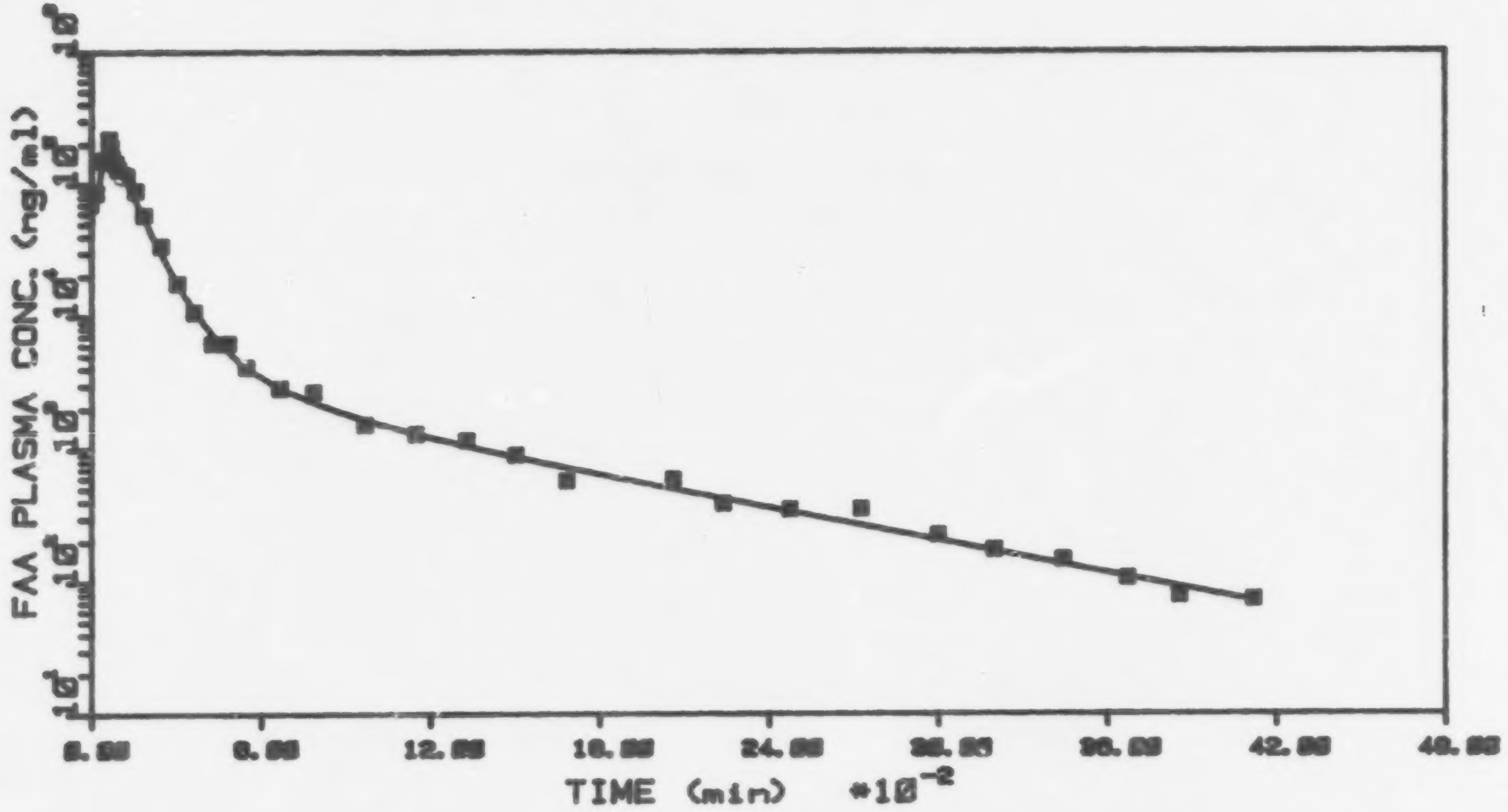
Fig. 8



PATIENT P.K.
DOSE = 2000 MG/METER SQ. FAA
CLEARANCE = 67.07 ML/MIN/M2

Fig. 9

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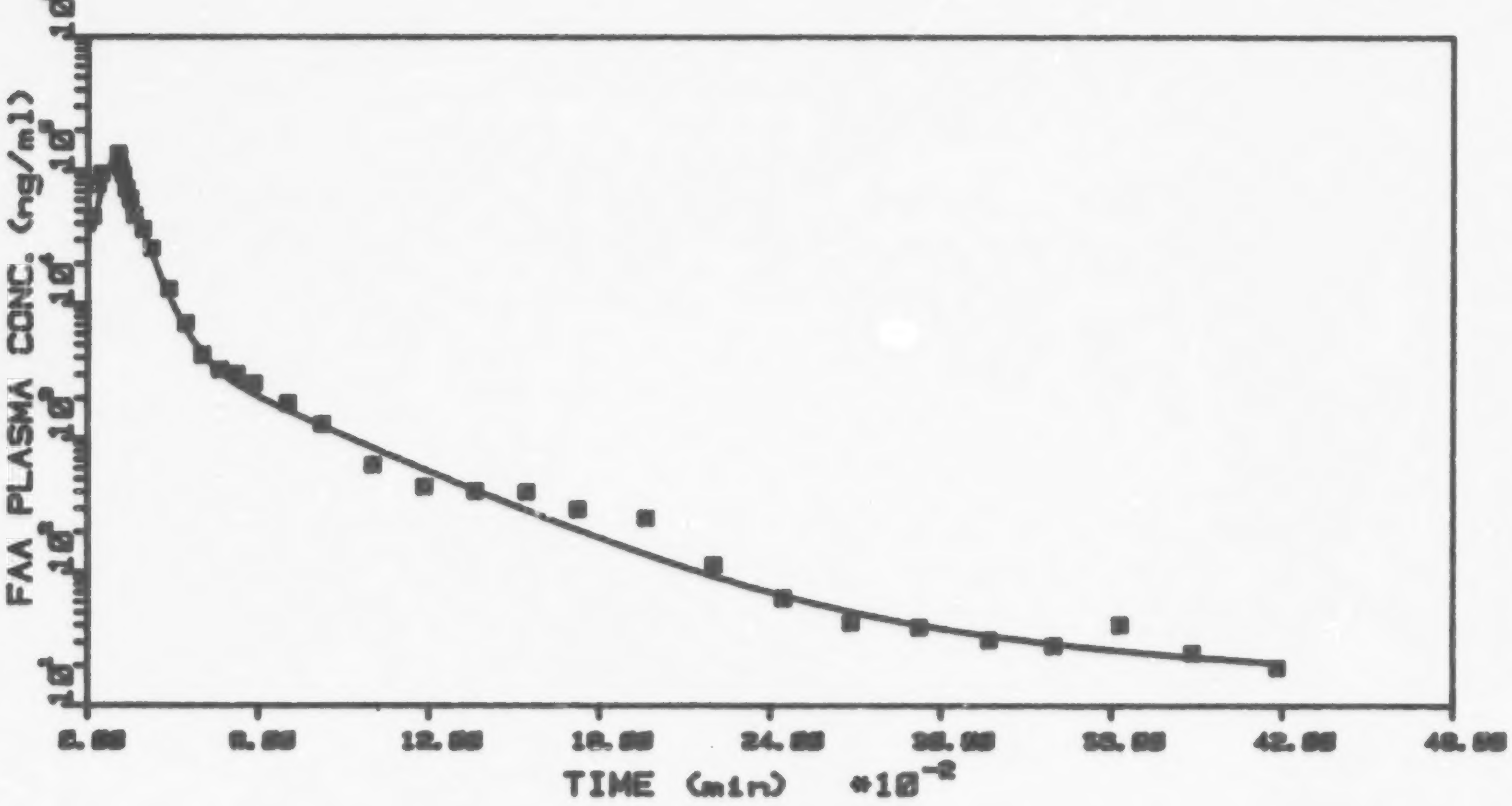
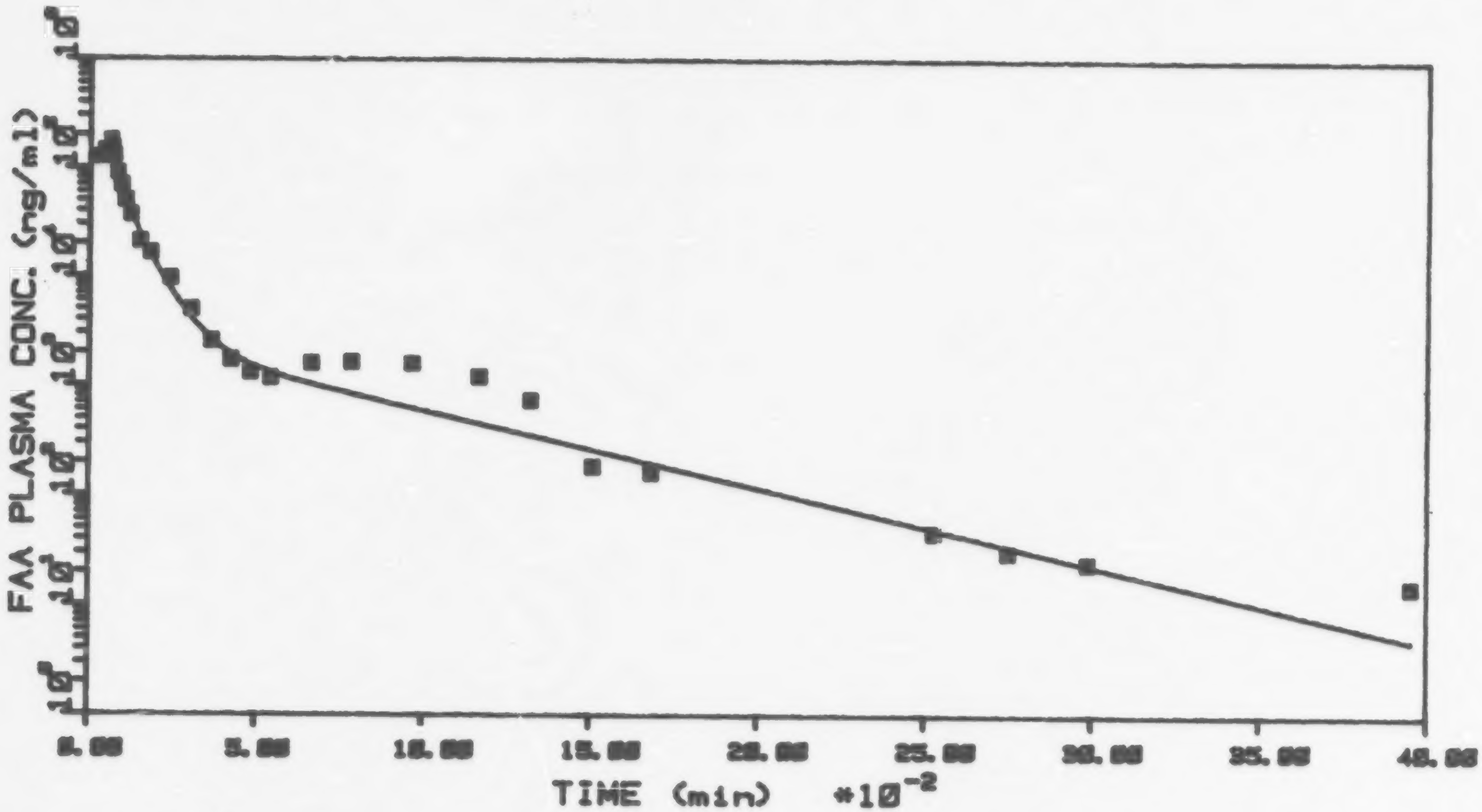


PATIENT G. K.
DOSE = 1500 MG/METER SQ. FAA
CLEARANCE = 76.78 ML/MIN/M2

Fig. 10



PATIENT N. V.
DOSE = 1500 MG/METER SQ. FAA
CLEARANCE = 96.33 ML/MIN/M2

Fig. 11

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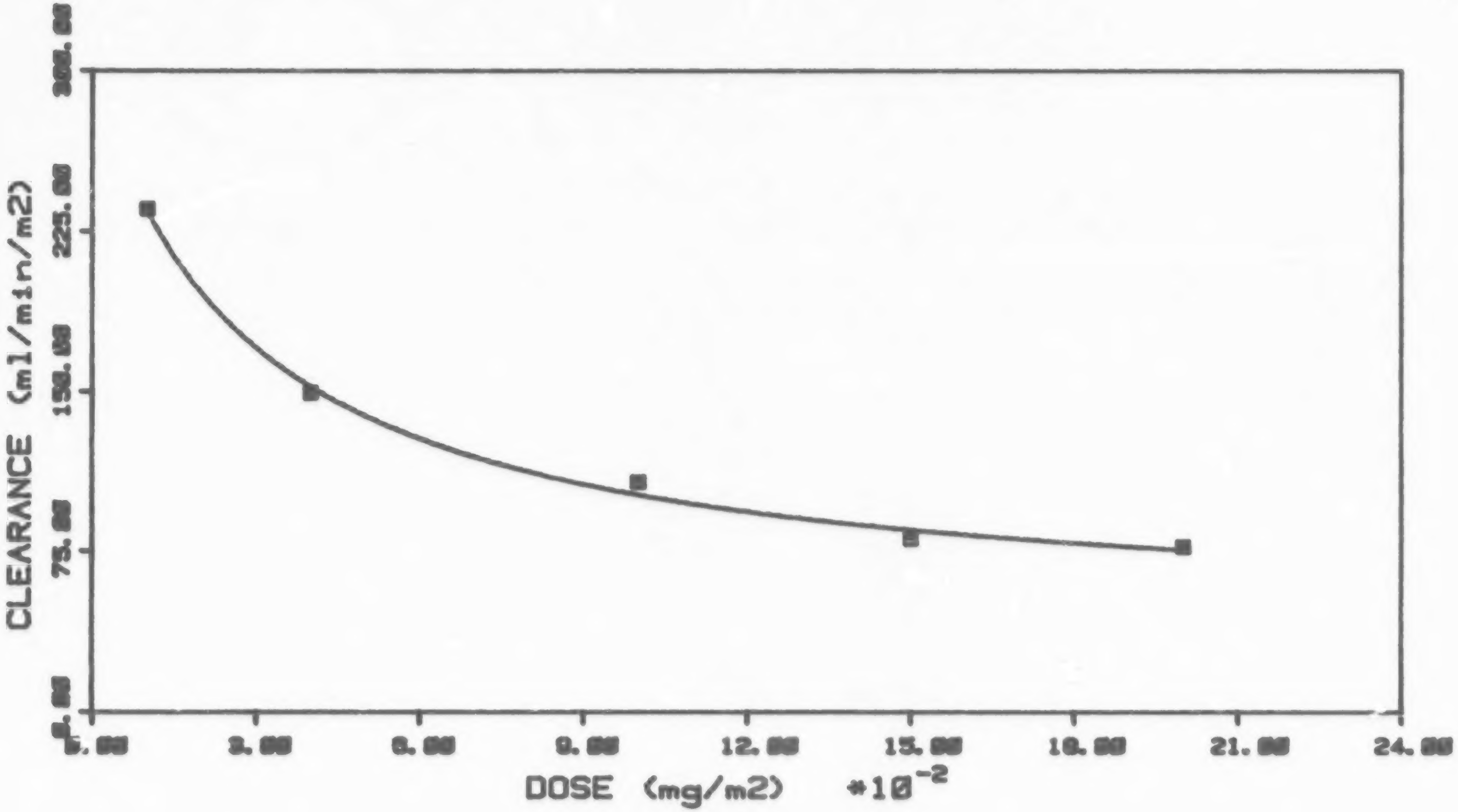
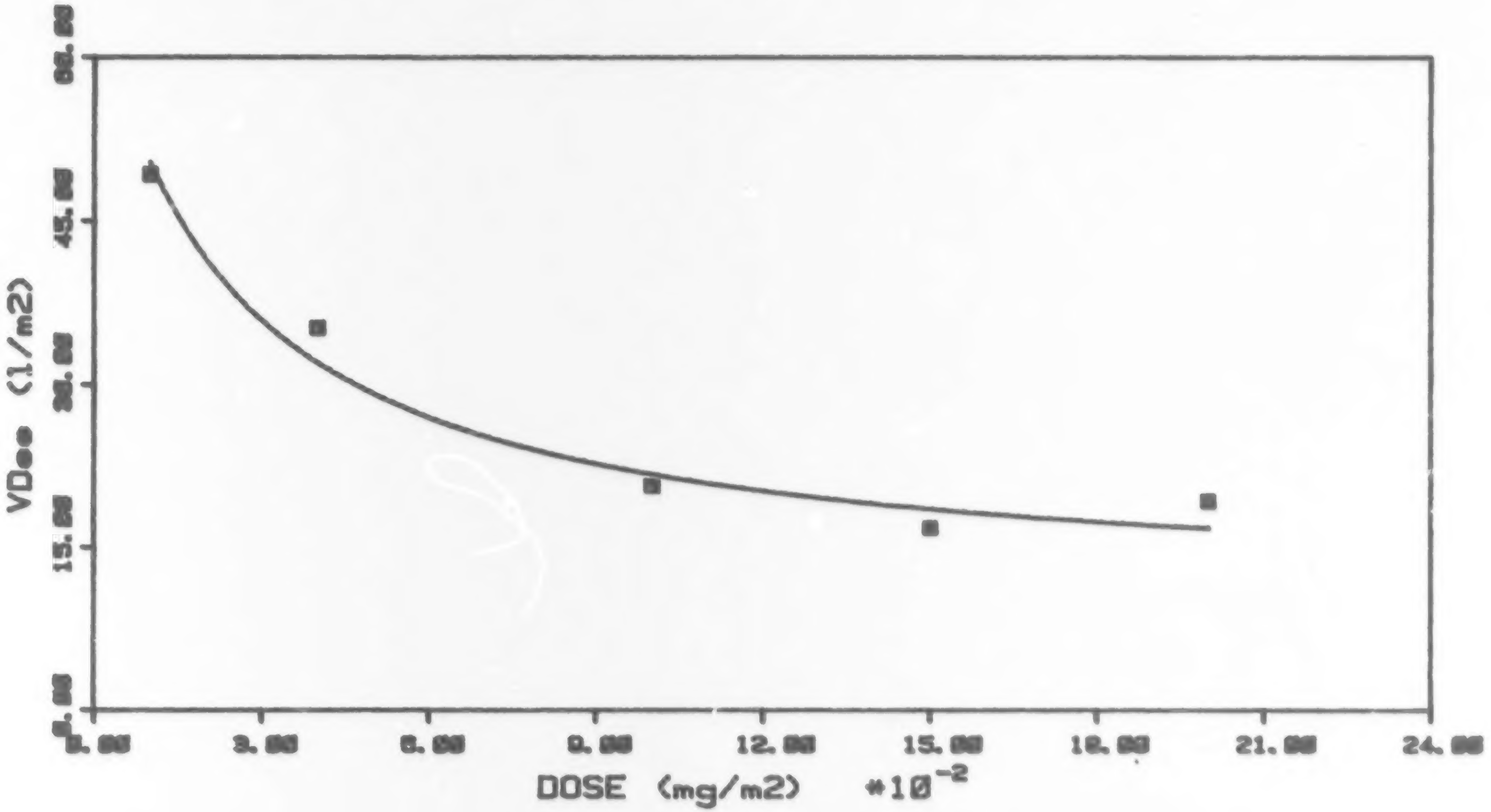


FLAVONE ACETIC ACID
MAXIMUM MEAN CLEARANCE = 297.78 ML/MIN/M2
MINIMUM MEAN CLEARANCE = 42.17 ML/MIN/M2

Fig. 12 Relation of FAA total body plasma clearance and dose.



FLAVONE ACETIC ACID
MAXIMUM MEAN VDss = 66.04 L/M2
MINIMUM MEAN VDss = 10.57 L/M2

Fig. 13 Relation of FAA steady state volume of distribution and dose.

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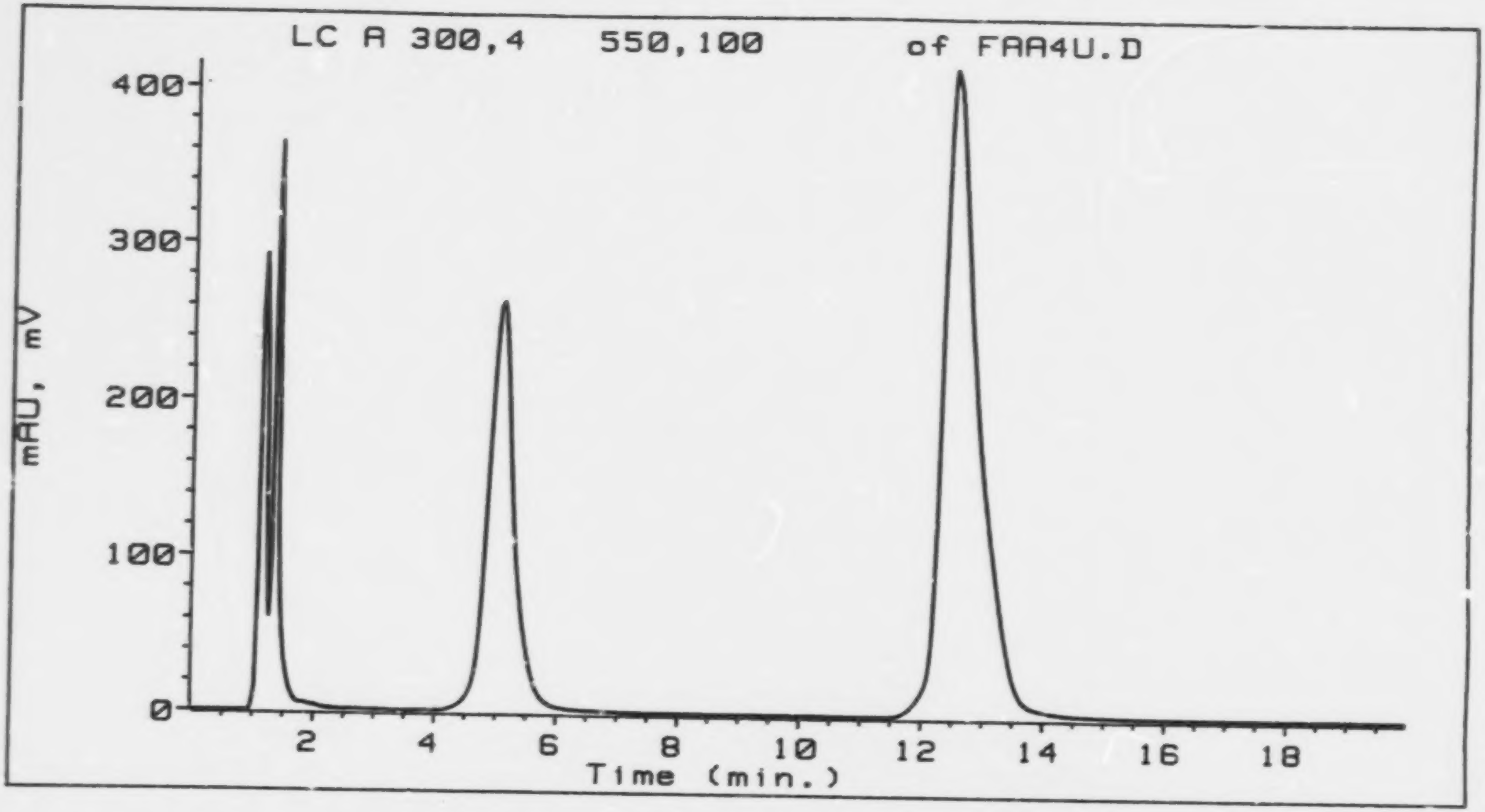

Fig. 14 Chromatogram of patient urine collected 0 - 1.25 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector at 300 nm. Chromatographic peaks: (5.1 min) metabolites, (12.4 min) FAA.

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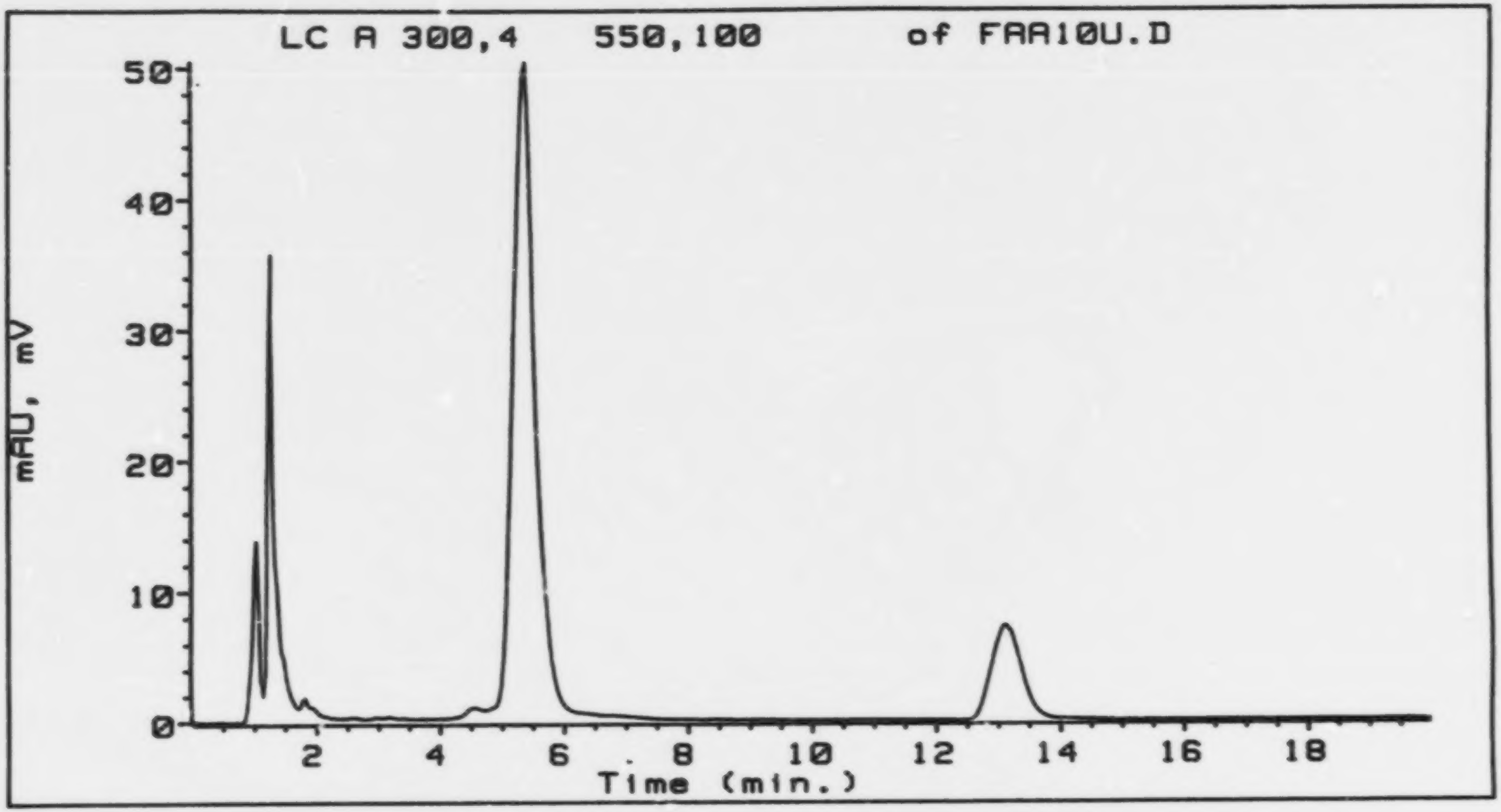

Fig. 15 Chromatogram of patient urine collected 10 - 11.6 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector at 300 nm. Chromatographic peaks: (5.3 min) metabolites, (13.2 min) FAA.

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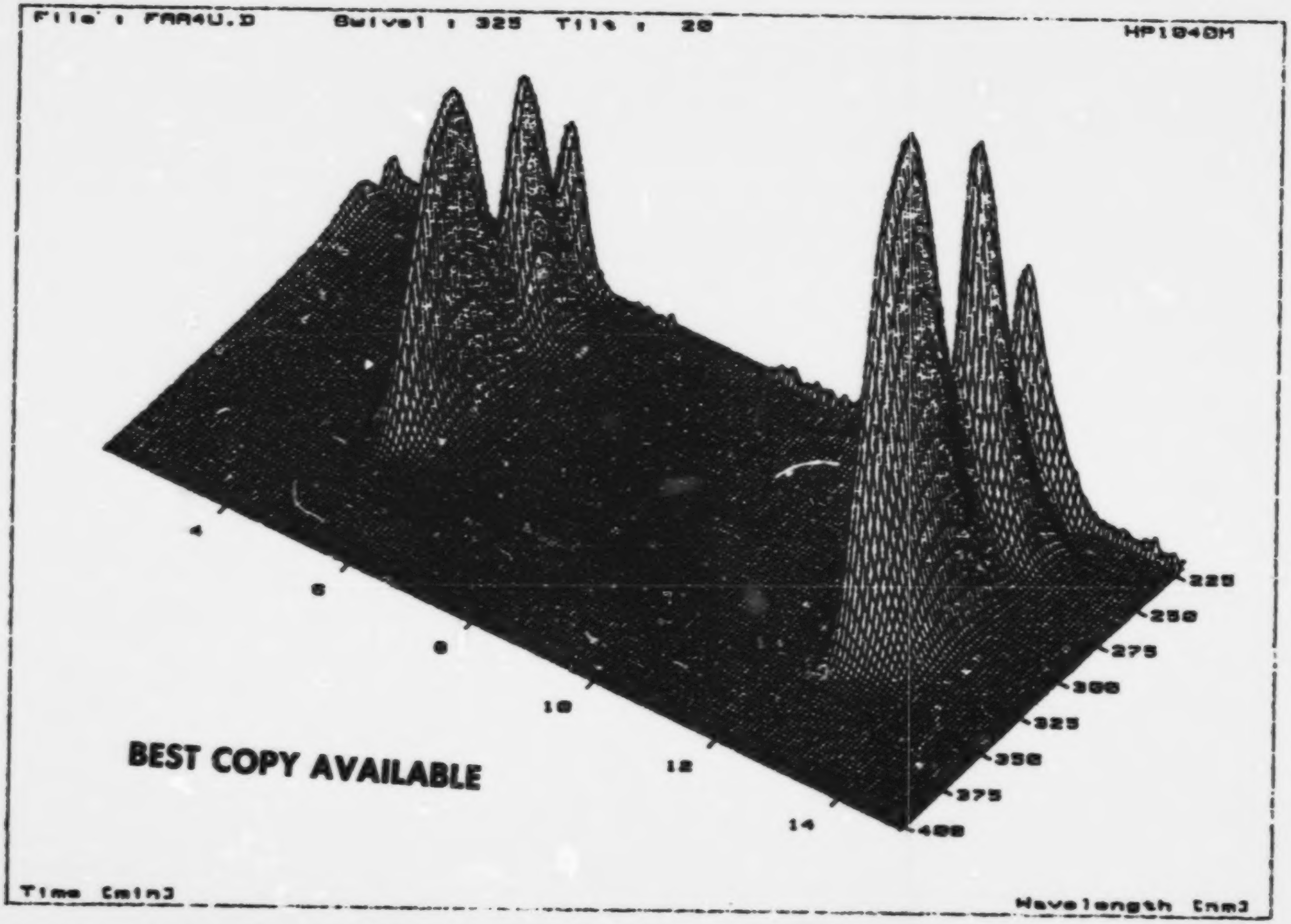

Fig. 16 Three-dimensional chromatogram of patient urine collected 0 - 1.25 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector. Chromatographic peaks: (5.1 min) metabolites, (12.4 min) FAA.

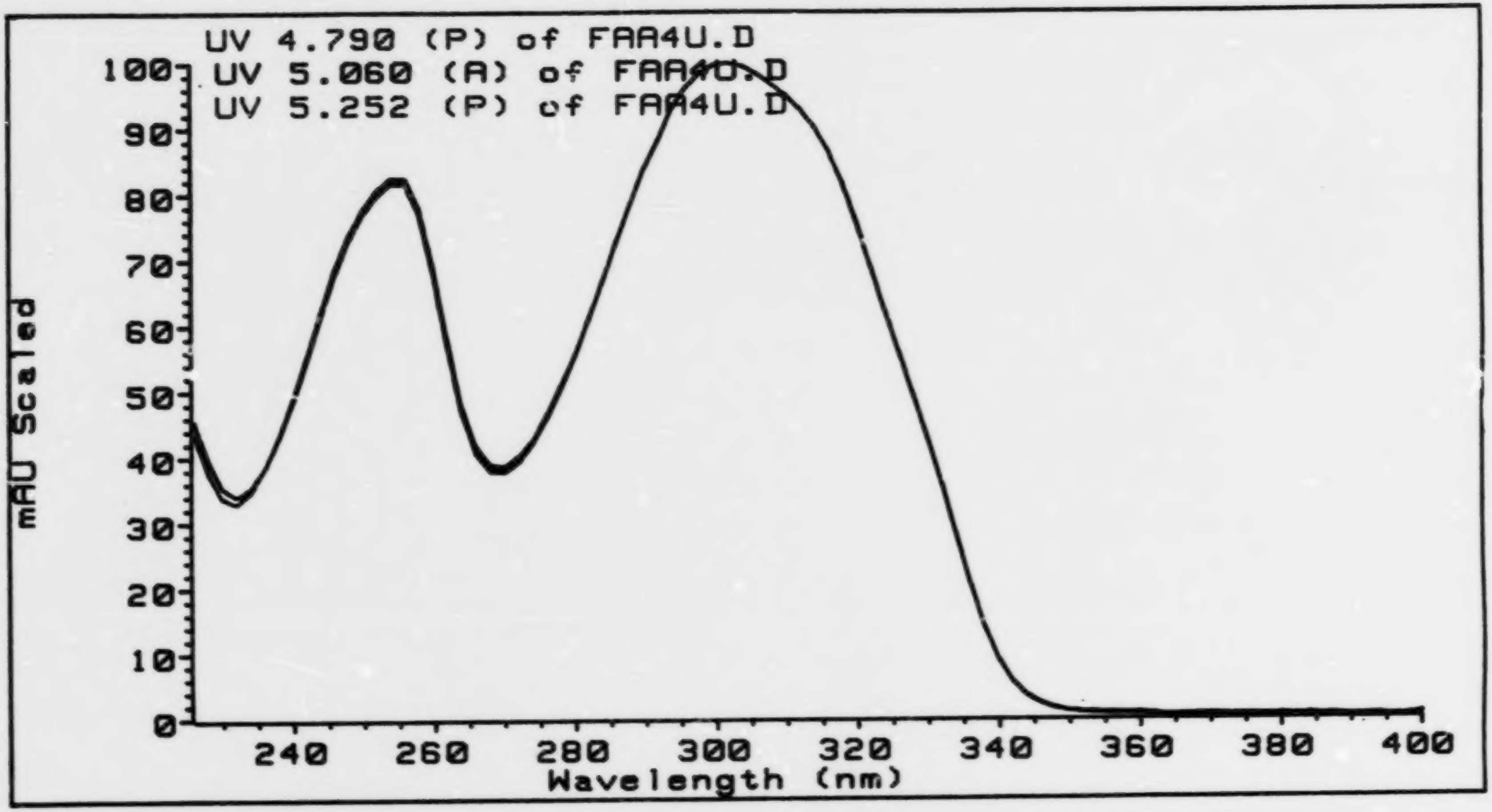

Fig. 17 UV spectra determined at the upslope, apex and downslope of the metabolite peak in a chromatogram of patiant urine collected 0 - 1.25 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2.

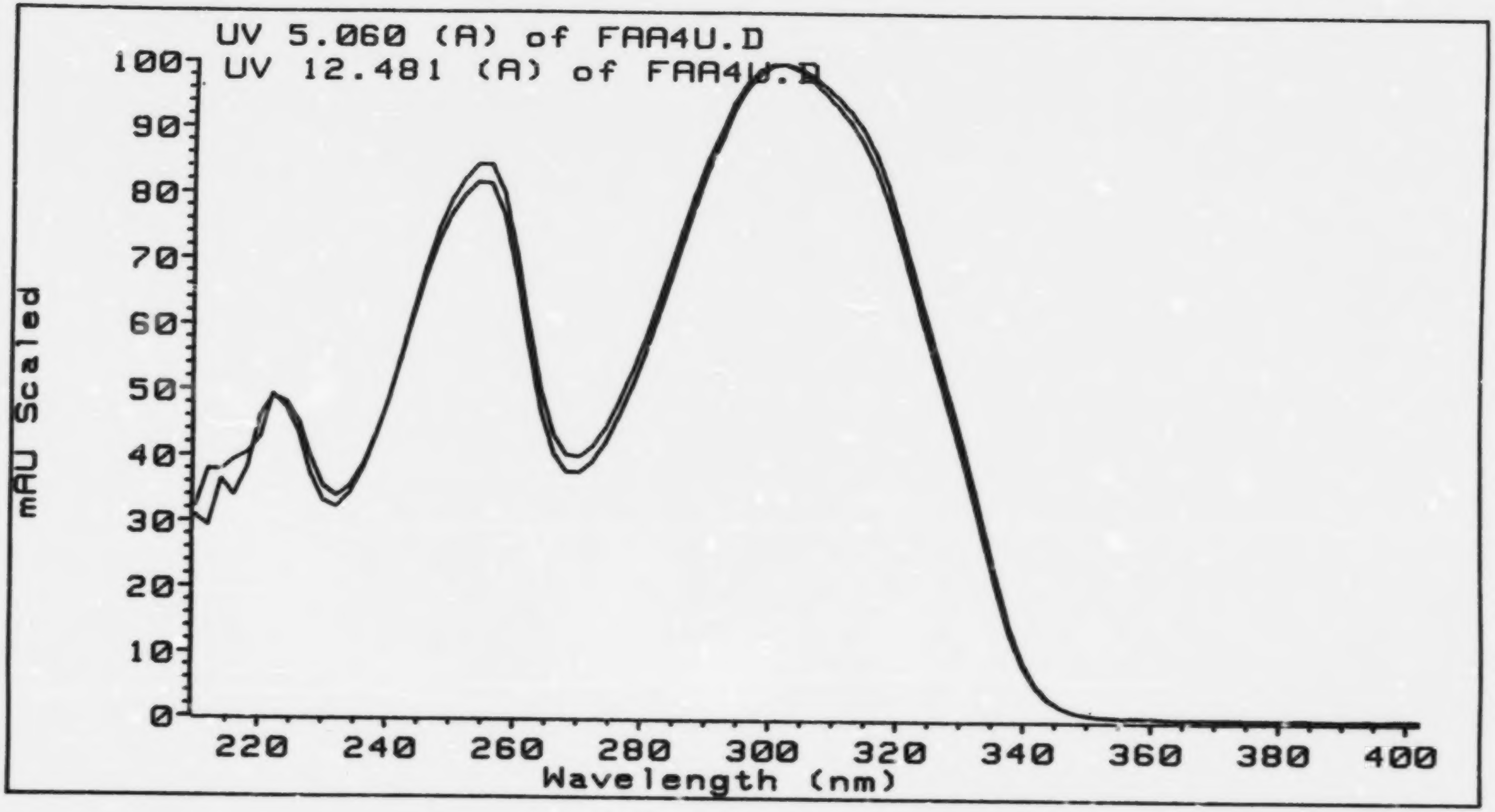

Fig. 18 Overlay of the UV spectra determined at the apex of the metabolite peak (5.1 min) and the FAA peak (12.5 min) in a chromatogram of patient urine collected 0 - 1.25 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2.

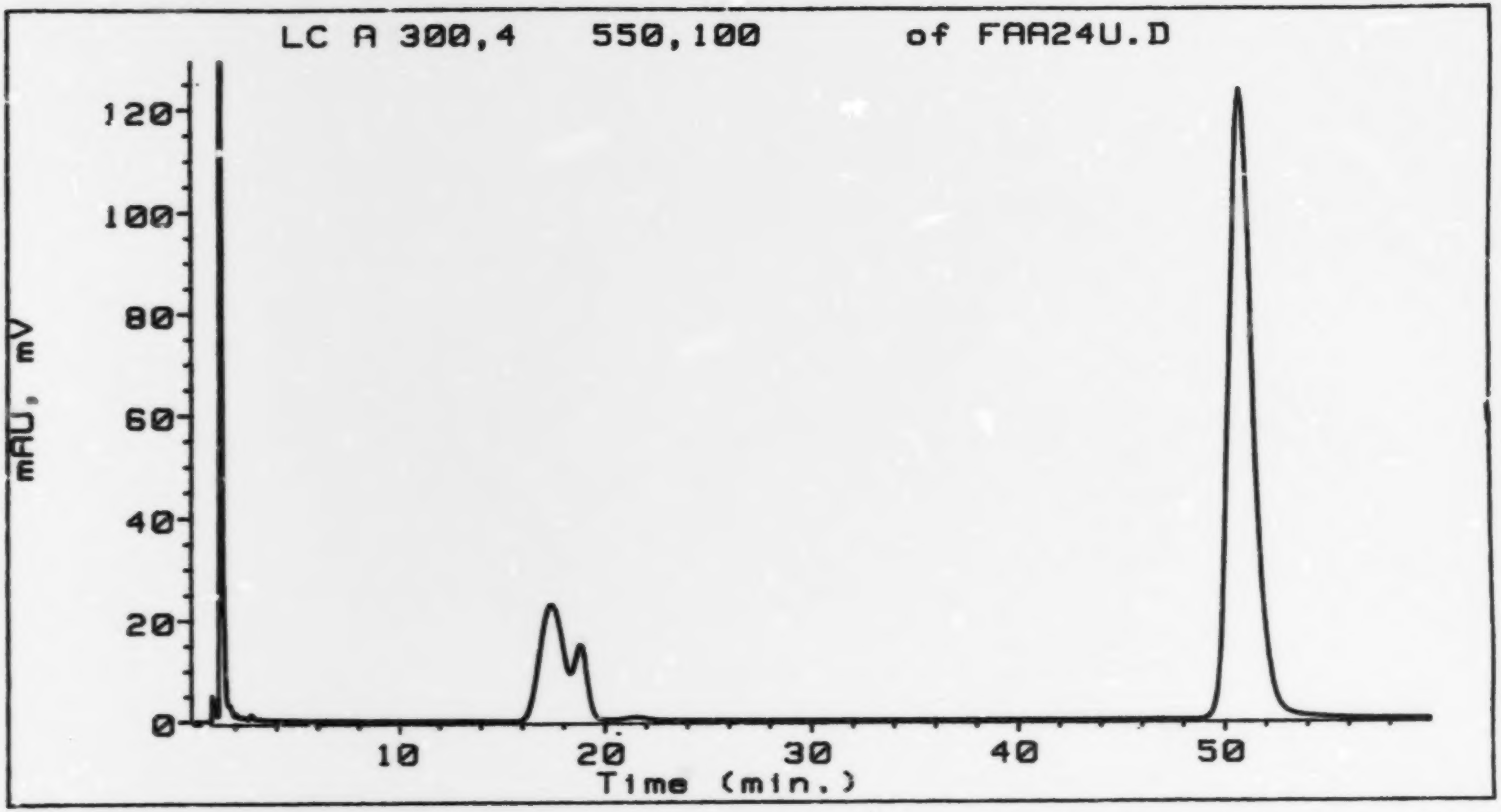

Fig. 19 Chromatogram of patient urine collected 0 - 1.5 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 determined with a diode array detector at 300 nm. The mobile phase was H_2O-MeOH-HAc, 68:30:2 v/v/v containing 0.001 M sodium dodecyl sulfate. Chromatographic peaks: (17.1 min) metabolite, (18.8 min) metabolite, (50.8 min) FAA.

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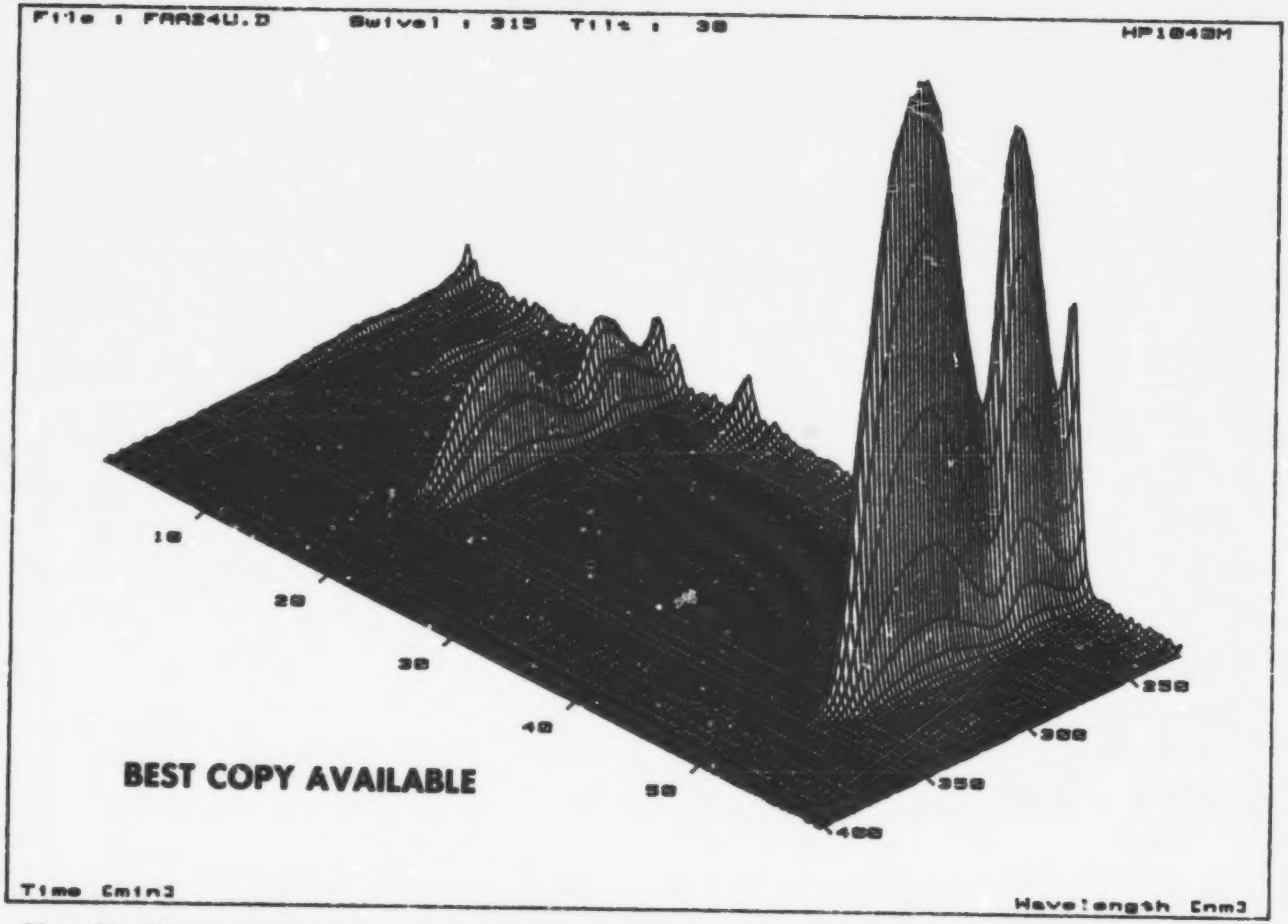

Fig. 20 Three-dimensional chromatogram of patient urine collected 0 - 1.5 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2 showing the metabolite peaks (17.1 min and 18.8 min) and the FAA peak (50.8 min). The mobile phase is described in Fig. 19.

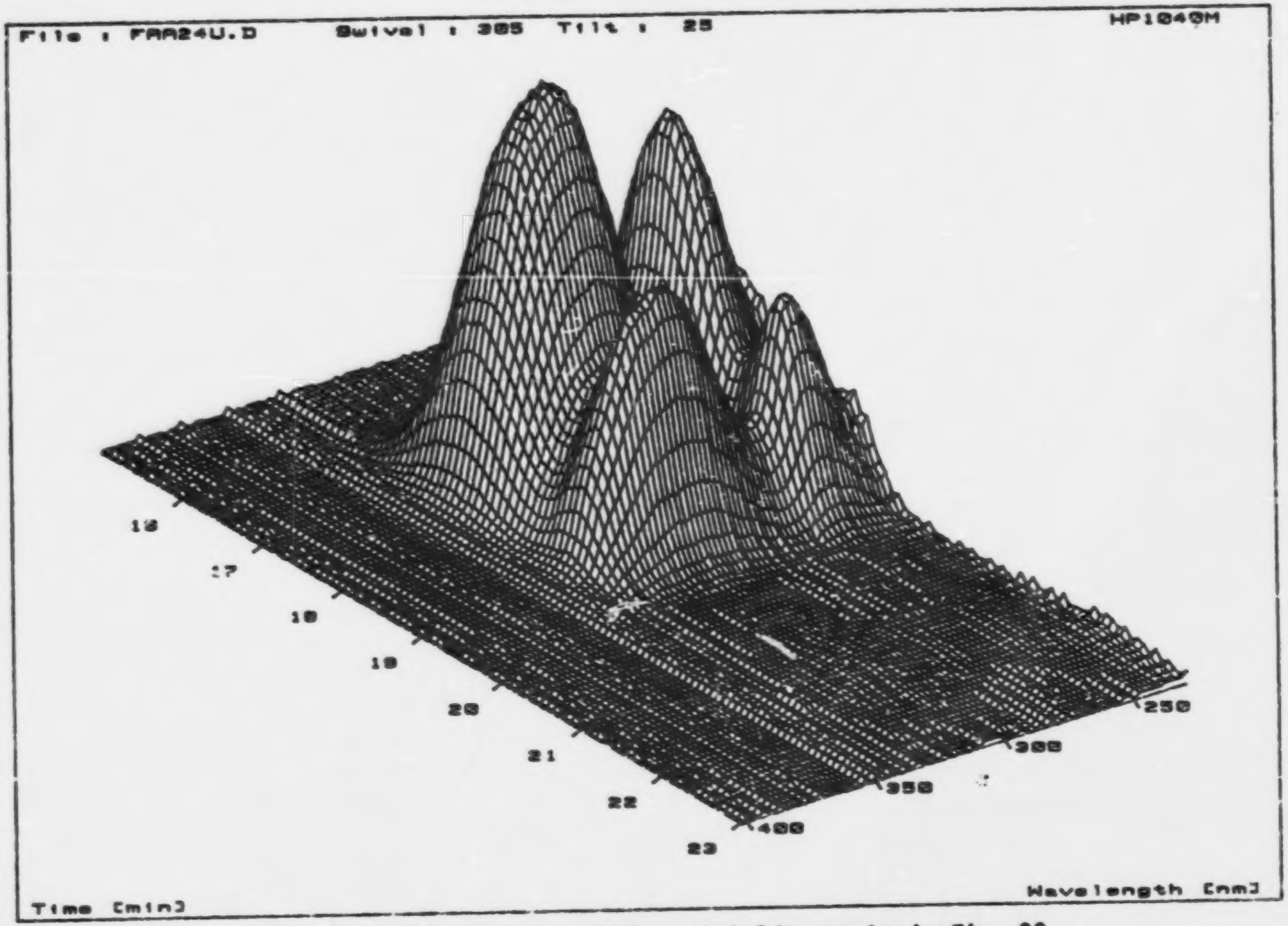

Fig. 21 Three-dimensional chromatogram of the metabolite peaks in Fig. 20.

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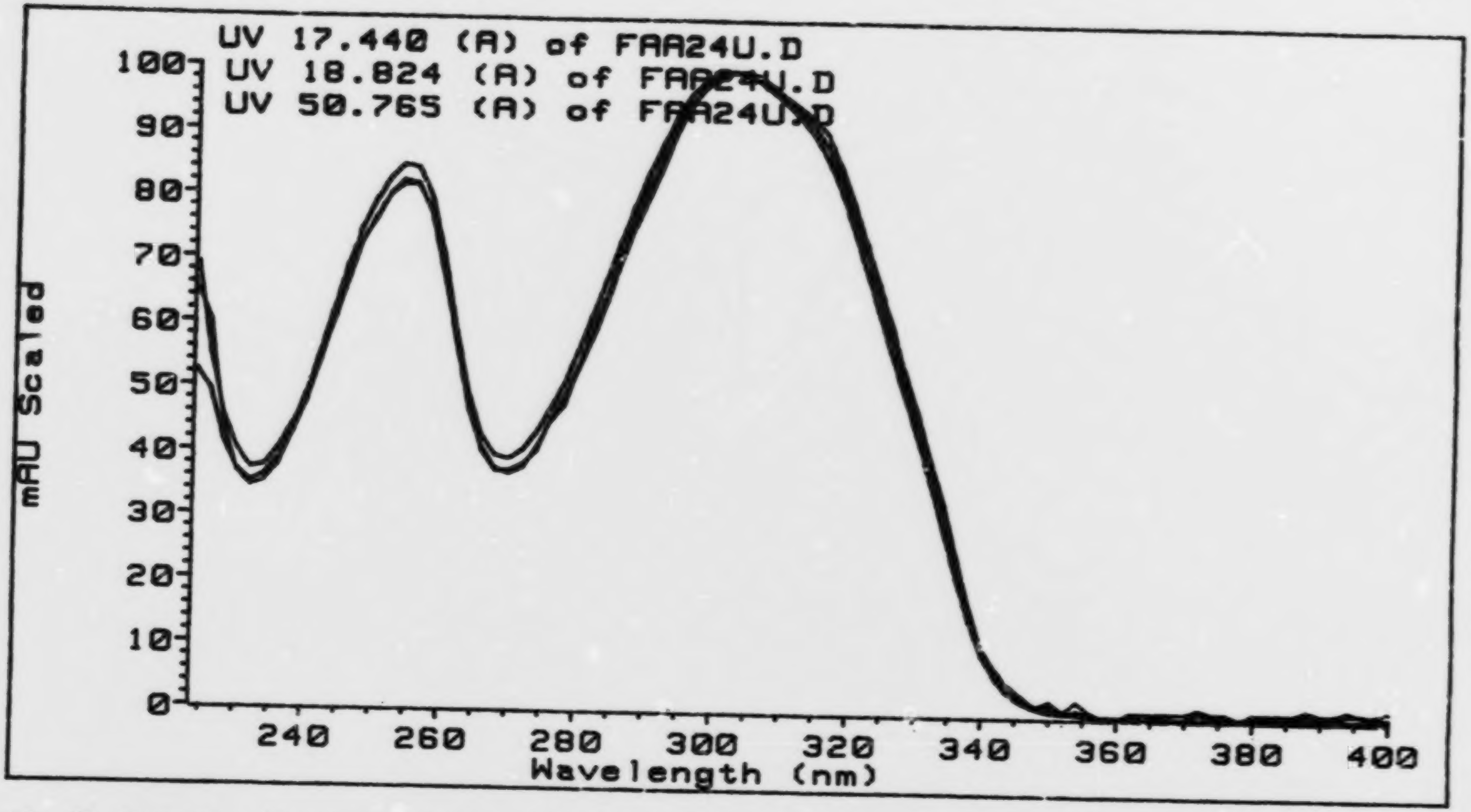

Fig. 22 UV spectra determined at the apex of the metabolite peaks (17.4 min and 18.8 min) and the FAA peak (50.8 min) in a chromatogram of patient urine collected 0 - 1.5 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2. Chromatograms are shown in Figs. 19-22.

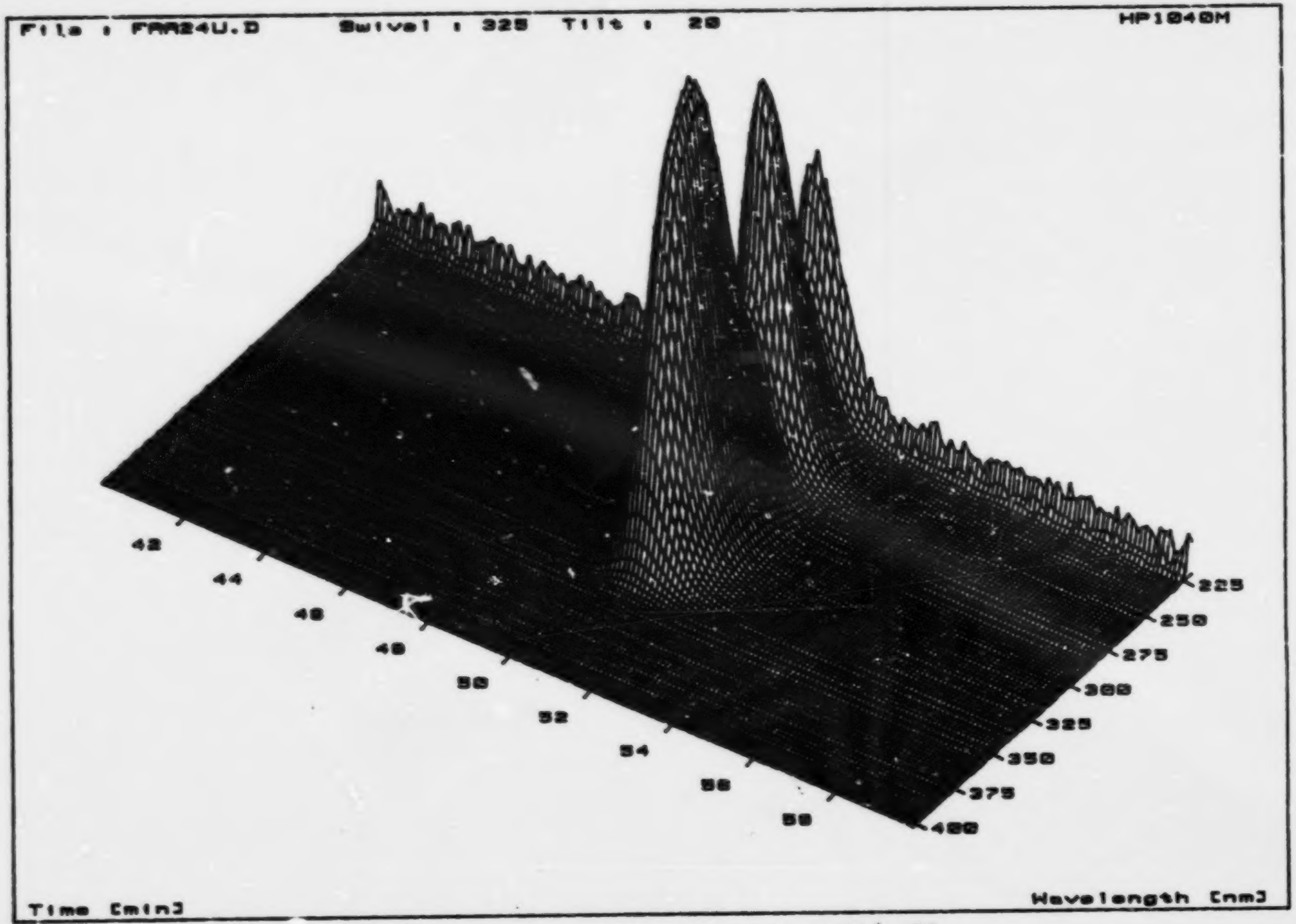

Fig. 23 Three-dimensional chromatogram of the FAA peak in Fig. 20.

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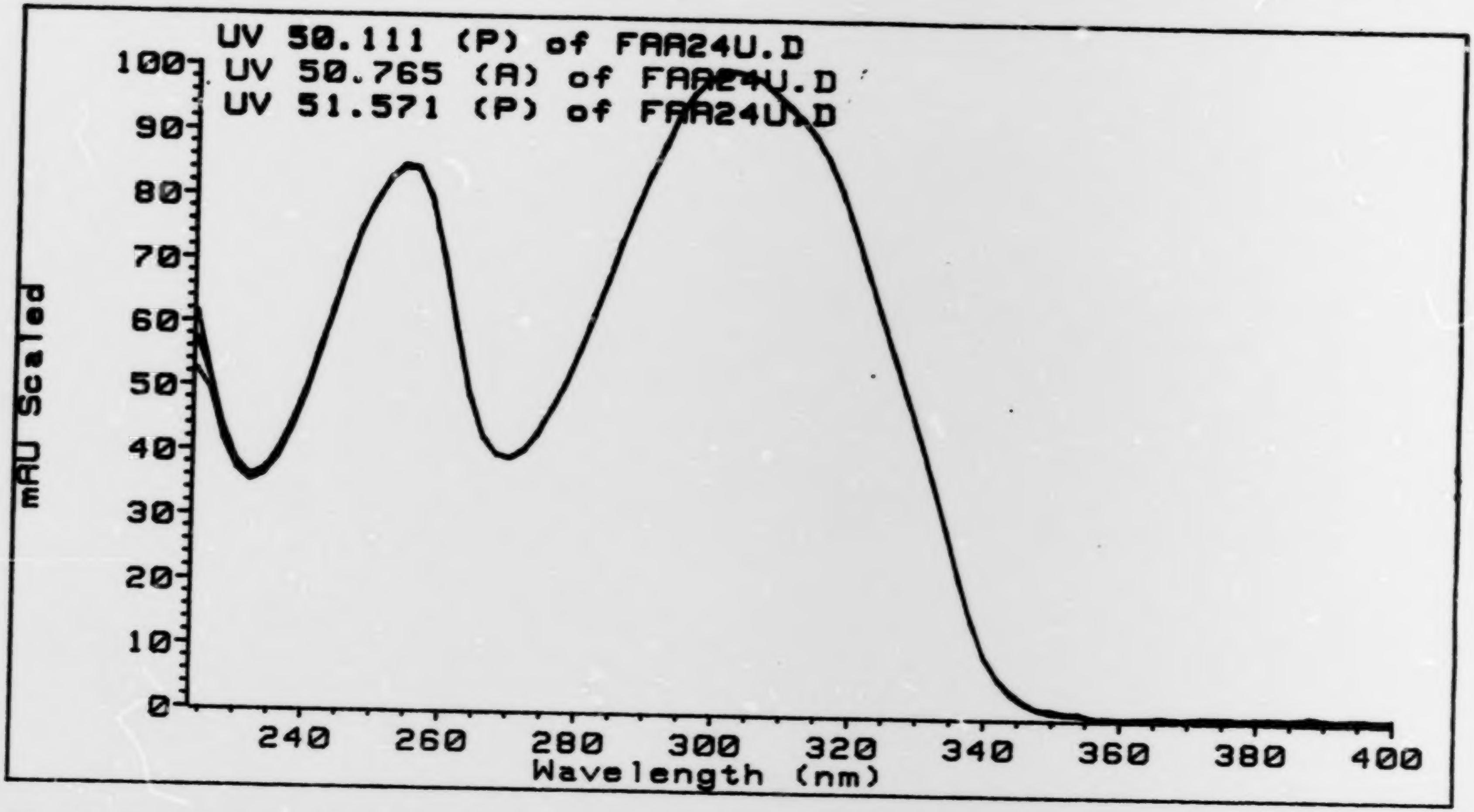

Fig. 24 UV spectra determined at the upslope, apex and downslope of the FAA peak in a chromatogram of patient urine collected 0 - 1.5 hr after the start of a one hour infusion of flavone acetic acid 2000 mg/m^2. Chromatograms are shown in Figs. 20 and 22.

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EORTC EARLY CLINICAL TRIALS GROUP

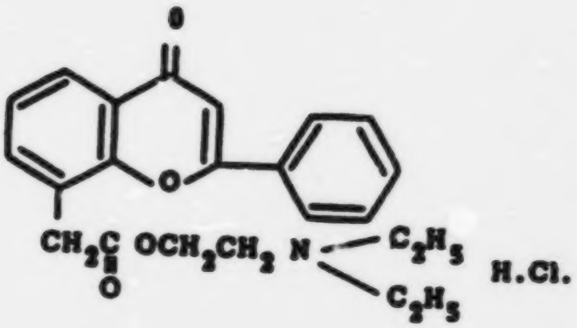
DRUGS EVALUATED IN PHASE I STUDIES (1985-1986)

Drug	Schedule	Center
TCNU	D X 1 Q 8-4 W	Copenhagen
	D X 1 Q 18-4 W P.O.	Edinburgh
Quinazoline	D X 1	Bellinzona
	D X 5	Amsterdam
	Weekly	Copenhagen
Phenylquinoline	D X 1	Amsterdam
	D X 5	Copenhagen
Mitozolomide	D X 1 Q 6 W	Bern

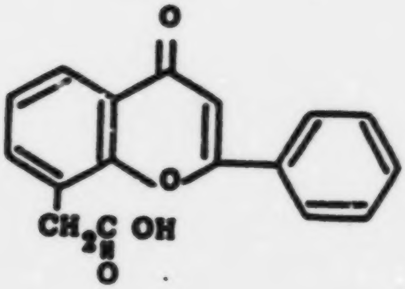
EORTC EARLY CLINICAL TRIALS GROUP

DRUGS EVALUATED IN PHASE I STUDIES (1985-1986) CONT.

Drug	Schedule	Center
LM 975	D X 1	Glasgow
LM 985	D X 1	Glasgow
	D1, 8 Q 4 W	Brussels
OC6020	Weekly	Amsterdam
Menogaril	D X 3 P.O.	Brussels
5-AZA-2'-Deoxycytidine	3 X D Q 5 W	Amsterdam
	D X 5 CI	Brussel/Pari
	D X 5 BOLUS	Nijmegen/Lyo
Trimelamol	D X 1 Q 3 W	Sutton
	D X 3 Q 3 W	Sutton


O
O
CH2C OCH2CH2 N
C2H5
C2H5
O
H.Cl.

LM985



LM975

Fig. 1. Structural formulae of LM985 and LM975.

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[CANCER RESEARCH 46, 3142–3146, June 1986]

Phase I and Pharmacokinetic Study of LM985 (Flavone Acetic Acid Ester)[1]

D. J. Kerr, S. B. Kaye,[2] J. Graham, J. Cassidy, M. Harding, A. Setanoians, J. C. McGrath, W. R. Vezin,
D. Cunningham, G. Forrest, and M. Soukop

Department of Medical Oncology [D. J. K., S. B. K., J. G., J. C., M. H., A. S.] and Institute of Physiology [J. C. M.], University of Glasgow, Department of Pharmacy, University of Strathclyde [W. R. V.], and Department of Medical Oncology, Royal Infirmary [D. C., G. F., M. S.], Glasgow, Scotland

ABSTRACT

We have conducted a Phase I and initial clinical pharmacological evaluation of LM985, the first of a series of compounds based on the flavone ring structure to be considered for clinical trial in malignant disease. The drug was administered i.v. to 26 patients with advanced cancer on an every-21-day schedule. Patients were treated at 14 dosage levels ranging from 10 to 1500 mg/m². Dose limiting toxicity was identified as acute reversible hypotension occurring during drug infusion; no leukopenia, alopecia, hepatic toxicity, or renal toxicity was observed, but at the higher dose range, mild sedation was apparent. Twenty patients had measurable disease and were evaluable for response. One patient with colorectal carcinoma had stable disease after three courses of LM985; however, no other responses were seen. Pharmacokinetic and *in vitro* drug degradation studies imply that the ester LM985 is hydrolyzed to LM975 (flavone acetic acid) rapidly *in vivo*. LM975 is active in a variety of animal tumor models, but it does not have the cardiovascular side effects seen with LM985 (hypotension and bradycardia) in pithed or anesthetized rats. We would recommend that *LM975* be considered for clinical trial, because it seems likely that substantially higher doses of LM975 than of LM985 can be given without dose limiting cardiovascular toxicity.

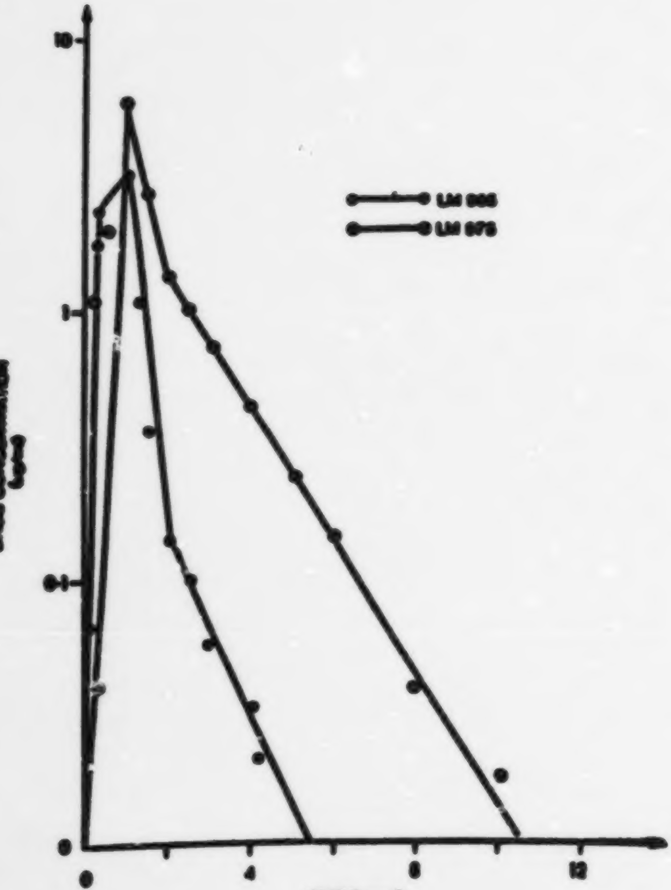

Fig. 1. Measured plasma concentration—time graphs of LM985 and LM975, following 1-h infusion of LM985 (600 mg).

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PHASE 1 TRIAL OF FLAVONE ACETIC ACID.

- STARTING DOSE OF 500 mg/m²
- 3 PATIENTS PER DOSE LEVEL.
- NO WITHIN PATIENT ESCALATION.
- 1 HOUR INFUSION, ONCE WEEKLY x 3.

PATIENT CHARACTERISTICS

Total : 23

M : 11
F : 12

Mean age : 53 yrs
Mean ECOG Performance Status : 2

Pathological diagnosis :	Colorectal carcinoma	10
	adenoca, unknown primary	6
	renal carcinoma	2
	other	5

Previous chemotherapy : 15

DRUG ADMINISTRATION

Intravenous infusion over 1 hour, diluted in 500ml of N-saline. In view of potential problems with urinary drug solubility at acidic pH, when administered in high dosage, we infuse sodium bicarbonate 1.26% (500ml) over 1 hour, immediately before and after administration.

SCHEDULE

Repeated weekly (x3), accoring to haematological parameters. If no evidence of disease progression at the end of the initial period, a further 3 courses are given, at weekly intervals.

SIDE EFFECTS

Transient nausea and vomiting noted in 2 patients ($1.5g/m^2$ and $2g/m^2$)

Flushing and a feeling of warmth has been a consistent feature at all doses > $1.5g/m^2$

No myelosuppression

No alopecia

No hepato-renal toxicity

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PHASE I STUDY OF LM975

Dose (gm/m^2)	No. of Patients	No. of Courses
0.5	3	11
1.0	3	10
1.5	3	18
2.0	3	18
2.7	4*	10
3.6	4**	12
4.8	3	9
6.4	1	1

* One patient : Early death (Disease)

** One patient : Urticarial rash during LM975

4th July 1986

DOSE (g/m^2)	PEAK CONC. (μg ml^{-1})	AUC (mg l^{-1}hr)	CLEARANCE (l hr^{-1})	Vd (l)	$t_{\frac{1}{2}}$ (hr)
0.5	53	85	11.9	56	3.8
1	67	125	11.8	42	2.5
1.5	125	242	9.5	46	3.4
2	168	446	8.1	84	6.7
2.7	213	585	8.6	60	6.5
3.6	442	1402	4.0	31	5.2
4.8	1174	3320	2.6	23	5.8

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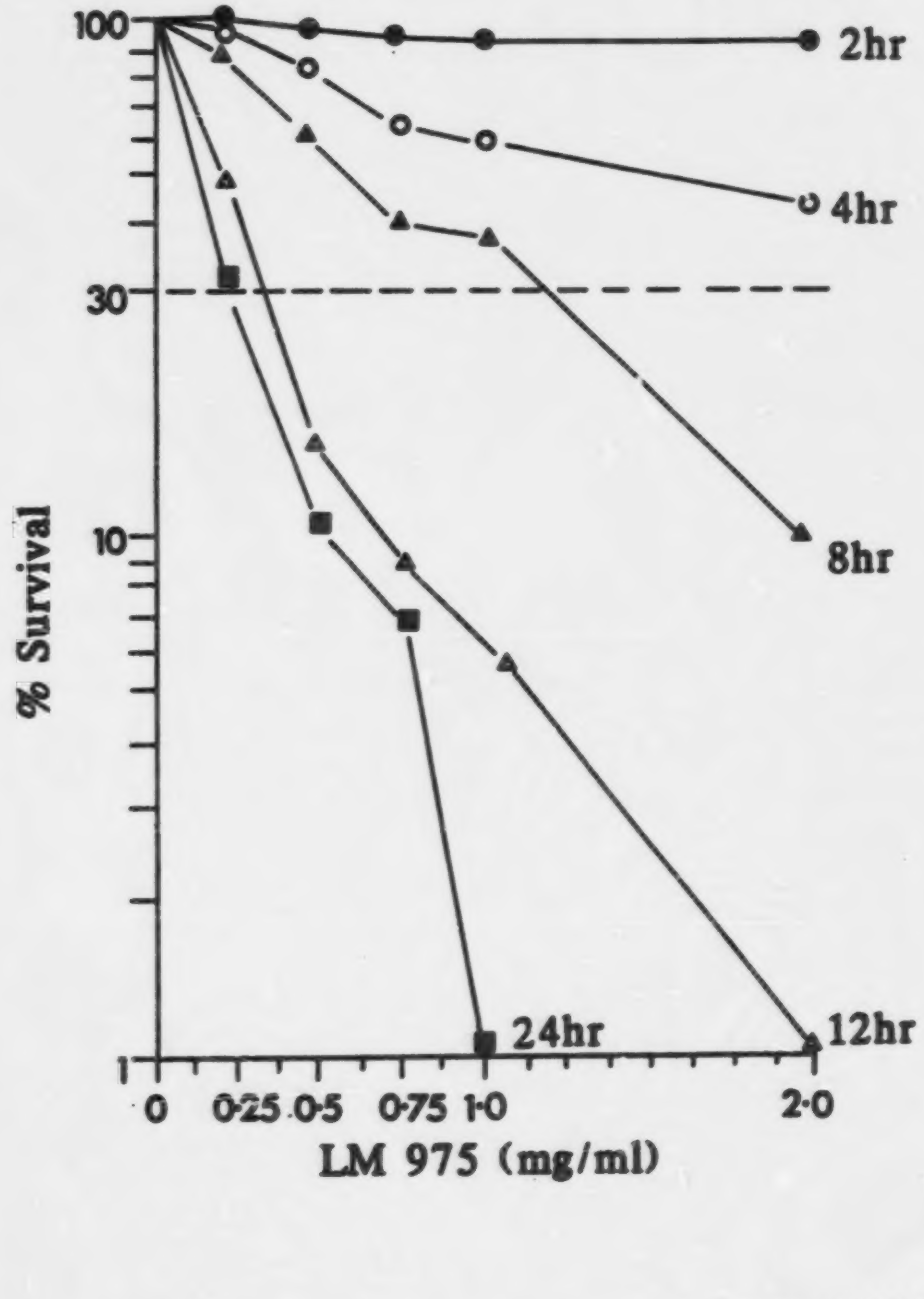
IN VITRO CHEMOSENSITIVITY OF MAC 15A TO LM 975



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PHASE I STUDY OF FLAVONE ACETIC ACID

SUMMARY:

- 24 Patients entered at doses of 0.5 to 6.4gm/m^2
- schedule is 1 hour infusion, once weekly x 3.
- major toxicity at highest doses is sensation of intense warmth, sweating, with some vomiting.
- no myelosuppression, alopecia, severe G.I. toxicity
- peak level reached at max. tolerated dose of 6.4gm/m^2 is 1936 ug/ml
- future patients to be entered at 6.4gm/m^2 as a 3 hour infusion.
- no response seen to date.

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Phase I Study of Acodazole

11/85

Schema:
25 mg/m^2
280 mg/m^2 I.V. over 1 hour every 21 days
360 mg/m^2
450 mg/m^2

Amended Dosing Levels:
560 mg/m^2 (skipped per NCI recommendation)
700 mg/m^2 I.V. over 1 hour every 21 days
875 mg/m^2
1095 mg/m^2
1370 mg/m^2

Accrual

	700	875	1095	1370
Entered	7	5	5	4
Eligible	7	5	5	4
Not Evaluable				
Refused	1*	-	-	-
Early Death	-	-	1**	-
Partially Evaluable - Toxicity				
Rapid Dx Prog	3	2	2	-
Early Death	1	1	-	-
Toxicity	-	-	-	3
Evaluable for Toxicity	6	5	4	4
Evaluable for Response	2	2	2	1

* Pt never received drug
** Expired day 5

Wayne State University
Phase I Study of Acodazole

Demographics

Previous Therapy

Chemo 34/37 = 91.89%
Horm 5/37 = 13.51%
Rad 26/37 = 70.27%
Surg 27/37 = 72.97%

Performance Status

Count	37
Mean	70.4
Median	70
Range	40 - 90

Metastases

Local	0
Soft Tissue	7
Bone	3
Visceral	27

Age

Count	37
Mean	58.
Median	59
Range	37 - 75

Sex

Male	17
Female	20

Primary Site/Cell Type

Lung	(9)
Adenoca	2
Squamous	4
Large	1
Oat	2

Sarcoma	(4)
Fibrosarc	1
Leiomyo	1
Mixed	1
Osteosarc	1

Colo-Rectal	(10)
Adenoca	10

STOMACH	(1)
Adenoca	1

ENDOMETRIUM	(1)
Adenosquam	1

BREAST	(8)
Adenoca	5
Infil Ductal	3

KIDNEY	(1)
Renal Cell	1

OVARY	(2)
Adenoca	1
Pap Cyst Adeno	1

PANCREAS	(1)
Adenoca	1

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Phase I Study of Acodazole

Hematologic Toxicity

		WBC				PLATELET		
Course	N	Mean	Median	Range	N	Mean	Median	Range
700 mg/m^2								
1	6	5.83	5.55	4.2-8.2	6	181.1	180	38-344
2	2	5.8	5.8	5.3-6.4	2	194.5	194.5	181-208
875 mg/m^2								
1	5	6.26	6.2	3.4-10.0	5	170	188	54-370
2	1	3.2	-	-	1	175	-	-
3	1	3.5	-	-	1	179	-	-
4	1	3.5	-	-	1	179	-	-
1095 mg/m^2								
1	4	6.8	7.1	2.8-10.2	4	312.7	361	254-378
2	1	6.4	-	-	1	271	-	-
1370 mg/m^2								
1	3	7.03	4.6	4.4-12.1	3	307	163	86-674

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Phase I Study of Acodazole

GI Toxicity

700 mg/m^2: One patient had moderate nausea and vomiting.

875 mg/m^2: One patient experienced mild nausea.
Two patients had moderate nausea and vomiting.
One patient experienced moderate to severe nausea and vomiting.

1095 mg/m^2: Two patients experienced mild nausea.
Two patients had moderate nausea and vomiting.

1370 mg/m^2: Two patient had moderate nausea and vomiting.
One patient had severe nausea and vomiting.

Other Toxicity

700 mg/m^2: One patient experienced mild to moderate hypertension.

One patient experienced mild lightheadedness and dizziness.

875 mg/m^2: One patient experienced mild to moderate itching after the completion of infusion.

1095 mg/m^2: One patient experienced mild burning in the chest.

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Phase I Study of Acodazole

Allergic Reactions

875 mg/m^2: One patient developed a life-threatening reaction after completion of drug infusion. Patient was intubated after anaphylatic shock.

1095 mg/m^2: One patient developed mild swelling of the lips after completion of infusion. Treated with antihistamines.

1370 mg/m^2: One patient developed a mild rash on neck approximately 15 minutes into infusion. Patient was treated with steroids and antihistamines. Administration of drug was continued by request of patient.

One patient developed a mild rash with itching in lower scalp and neck region. Patient treated with antihistamines and steroids. Patient refused continuation of drug infusion, only 50 mg of Acodazole was infused.

CARDIAC TOXICITY

1370 mg/M^2 "Palpitations and Fluttering of Heart" 50 minutes into infusion noted to have unifocal PVCs. Infusion terminated. PVCs continued for approximately 1½ hours post infusion.

1370 mg/M^2 PVCs noted near conclusion of infusion. QT prolongation noted. Associated with hypokalemia.

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T84-0197
WSU
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Alterations in Electrolytes & QTC

1.4-2.4 8.5-10.5

TIME(Minutes)	BP	HR	QT	QTC	K	Mg	Ca^{++}	Drug Level(mcg/ml)
0	108/64	90	0.34	0.43	3.2	1.7	8.7	0
15	114/68	92	0.40	0.51	3.1	1.7	8.7	14.3
30	104/60	96	0.40	0.51	4.0	1.6	9.1	21.7
end of infusion 45	130/70	106	0.42	0.52	3.0	1.6	8.6	23.30
60	118/70	104	0.40	0.54	3.3	1.5	8.4	-
Post Infusion								
15	134/68	104	0.44	0.60 significant ↑	2.6	1.4	7.7	7.62
30	110/66	96	0.46	0.59	2.8	1.3	7.1	6.28
45	120/60	90	0.44	0.50	2.7	1.4	7.5	5.4
60	110/60	82	0.48	0.57	-	-	-	5.01
4 Hours		88	0.48	0.54	3.2	1.4	8.0	2.85
6 Hours		74	0.42	0.47	3.2	-	-	2.35

Alterations in Electrolytes & QTC

Dose: 1370 mg/M^2 over 1 hour infusion

Pt received 425cc = 1908 mg

Infusion started on 1/16/86 at 9:30 am Infusion Ended 10:22 am

Infusion was stopped after 425 of 500cc (1908 mg) was infused because of QT prolongation and onset of frequent unifocal PVC's (bigeminy)

QTC = QT interval Corrected for heart rate

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PROPOSED ANIMAL STUDY

- Anesthetized adult mongrel dogs with catheters placed in carotid artery and jugular vein. Standard EKG leads.
- Electrolytes, Mg^{++}, Ca^{++}, hemodynamics (HR, BP, LAP, LV dP/dt) and 2D echos recorded pre-infusion of Acodazole, then 15, 30, 45, 60 minutes into infusion. Repeated after infusion.
- Initial dose 700 mg/M^2

Acodazole
T83-1071
Wisconsin
July 1986

PATIENT CHARACTERISTICS

Acodazole, 1hour infusion, weeklyX4

SEX	16 female 25 male
MEDIAN AGE	60 (32-77)
PERFORMANCE STATUS	0--13 I--25 II--4
PRIOR THERAPY	NONE--3 CHEMOTHERAPY--16 XRT--1 XRT+CHEMO--19
TUMOR TYPES	COLORECTAL--16 LUNG--5 OTHER--10

STUDY DESIGN

1. The starting dose for this phase I study was 1/10 the MELD 10: 20mg/sq m/week X4

2. The acodazole dose was given in 100cc of 0.9% saline, infused over one hour with a "piggy back" infusion of 250cc of 0.9% saline for patients in whom irritation at the infusion site occurred.

3. Infusions were repeated weekly X4 and treatment cycles were repeated every six weeks.

4. Blood pressure and pulse were measured every 15 minutes during the infusion.

5. Weekly CBC, liver function tests and creatinine were measured.

INITIAL RESULTS

Escalation of dose from 20mg/sq m/week X4 through 888mg/sqm/week-X4 was accomplished. Only mild toxicity was recognized.

Dose	no.patients	courses	nausea/vomiting (> grade II)	venous "flare"
20mg/sq m	3	6	0	4/6
40mg/sq m	3	5	0	3/5
65mg/sq m	3	6	0	2/6
100mg/sq m	3	8	0	7/8
150mg/sq m	3	6	0	5/6
225mg/sq m	3	3	1/3	3/3
340mg/sq m	4	3	3/3	3/3
500mg/sq m	3	3	0	2/3
666mg/sq m	3	4	4/4	1/4
888mg/sq m	5	7	7/7	4/7
	33	51		

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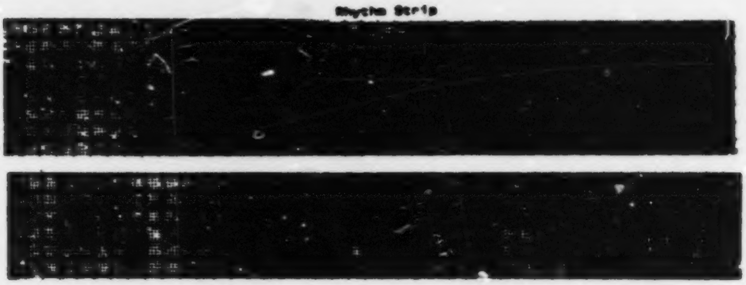
MYELOSUPPRESSION

Dose	Nadir WBC(median)	Nadir platelet(median)
20mg/sq m	4450/cumm	185,000/cumm
40mg/sq m	7300	271,000
65mg/sq m	8350	424,000
100mg/sq m	7150	373,000
150mg/sq m	8750	547,000
225mg/sq m	9100	404,000
340mg/sq m	9900	518,000
500mg/sq m	10,300	447,000
666mg/sq m	6700	217,000
888mg/sq m	3400*	220,000

*in 3/7 courses WBC nadir was less than 3000/cumm but greater than 2500/cumm

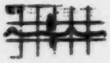
ELECTROCARDIOGRAPHIC FINDINGS IN THE FIRST PATIENT AT 1184 mg/sq m

Rhythm Strip



Lead II

Pre-therapy
QT interval=0.359



90 min.
QT interval=0.480sec

10 hr.
QT interval=0.514sec

24hr.
QT interval=0.44sec

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Two hours after the completion of the second infusion of acodazole in the first patient at 1184mg/sq m the patient's pulse was noted to be irregular. "Runs" of polymorphic ventricular tachycardia in conjunction with a prolonged QT interval were noted.

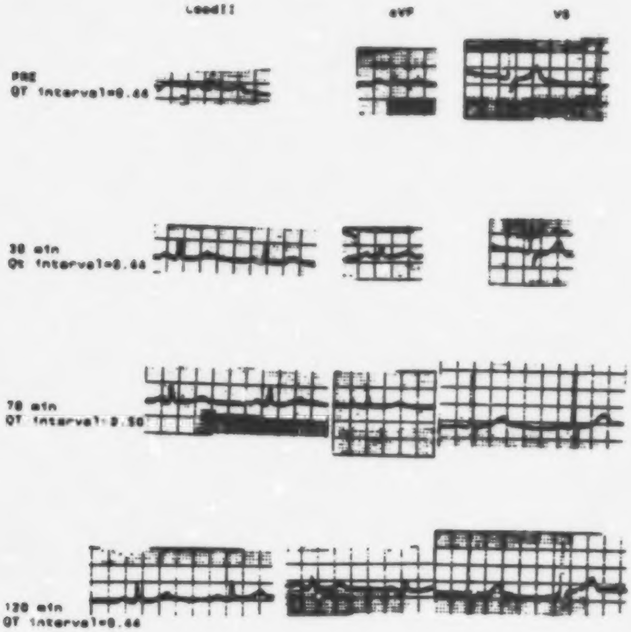
Lidocaine was administered(with equivocal benefit). The arrhythmia and QT prolongation resolved over 8-24 hours.

The dangerous arrhythmias seen in this patient were not due.to altered clearance of acodazole.

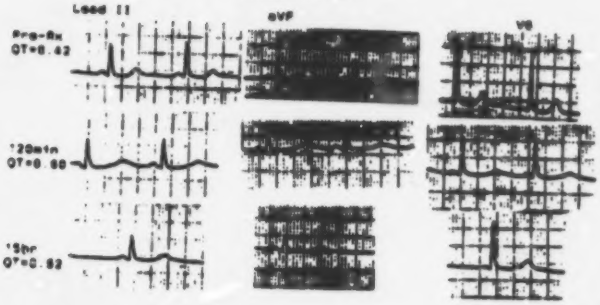
Time	Observed acodazole concentration	Predicted
30 minutes	11.3mcg/ml	12.5mcg/ml
12.5 hr	1.14mcg/ml	1.33mcg/ml
25.3 hr	0.75mcg/ml	0.79mcg/ml

ECG tracings were obtained in two other patients receiving 888mg/sq m. Substantial QT prolongation without ventricular arrhythmias were noted in both patients.

PATIENT 1


Lead II
aVF
V6
PRE
QT interval=0.44
30 min
QT interval=0.44
70 min
QT interval=0.50
120 min
QT interval=0.44

PATIENT 2


Lead II
aVF
V6
Pre-Rx
QT=0.42
120min
QT=0.60
15hr
QT=0.52

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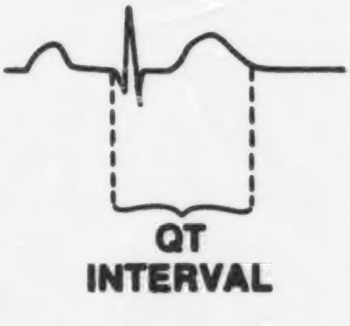
SUBSEQUENT PLAN OF INVESTIGATION

In order to investigate the frequency and extent of QT prolongation and arrhythmias, the following plan was carried out:

1. Five patients were treated on this schedule:
    - Week one--340mg/sq m
    - Week two--500mg/sq m
    - Week three--666mg/sq m
    - Week four--888mg/sq m
2. Frequent ECG monitoring was carried out
3. Eligibility criteria were altered
    - a. normal ECG before therapy
    - b. no prior history of heart disease
    - c. normal electrolytes,magnesium,calcium
    - d. receiving no phenothiazines or drugs for cardiac disease

QT INTERVAL

The QT interval represents the phase of ventricular repolarization and is a period of vulnerability to dangerous arrhythmias.



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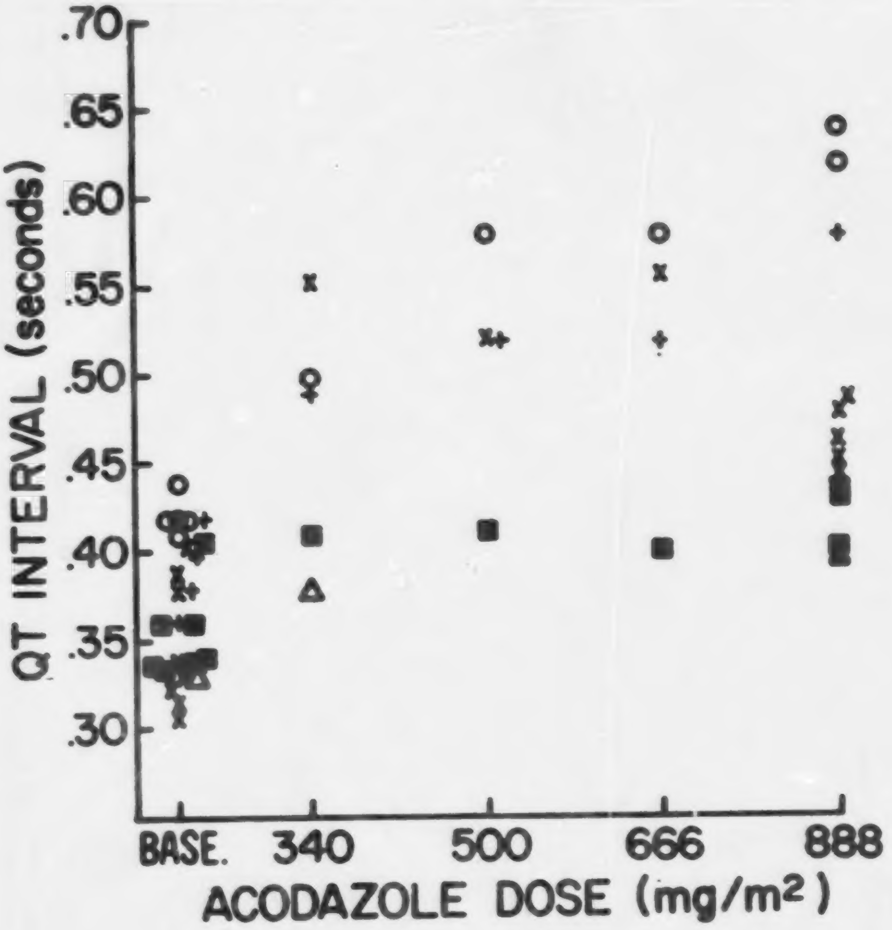
QT INTERVAL
PRE THERAPY AND MAXIMUM POST THERAPY
(5 patients, 24 courses)



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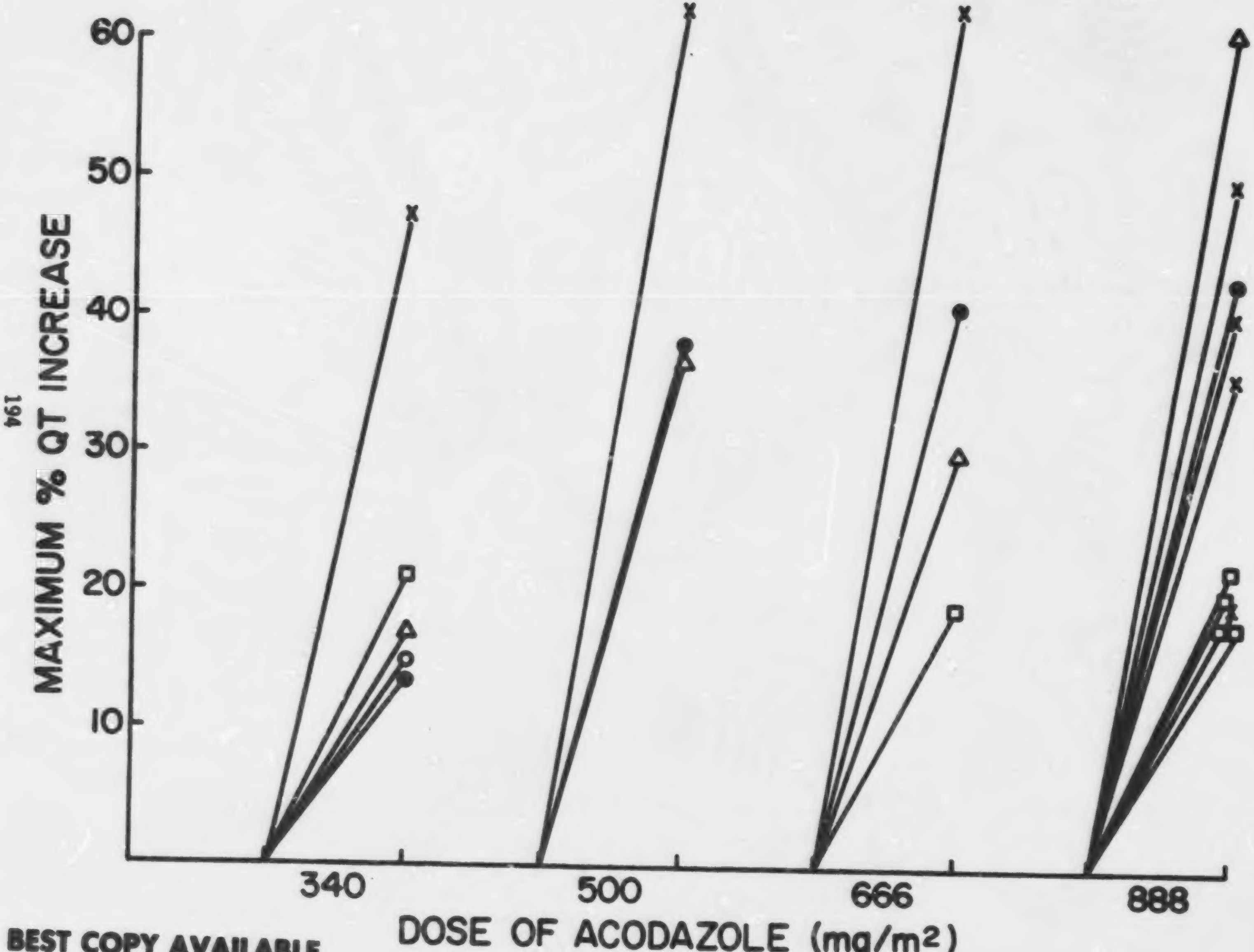
MAXIMUM INCREASE IN QT INTERVAL (5 patients, 24 courses)



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FINDINGS

1. QT interval prolongation(>20% above baseline) occurred following 21 of 27 courses of acodazole at doses >340mg/sq m. Bradycardia was also seen at each dose level >340mg/sq m. QT interval,corrected for decreased heart rate, was also increased by acodazole(27/27 courses). No clearcut dose-response relationship was demonstrated at the doses studied.
2. Other ECG intervals (PR,QRS) were unchanged.
3. Serum magnesium,calcium,and potassium did not change during and for 3-4 hours following the completion of acodazole infusion.
4. Peak acodazole plasma concentrations in patients in whom ECG changes were documented were not different from those seen in other patients.

SIGNIFICANCE

1. QT interval prolongaton is common after each acodazole administration and may be associated with life-threatening arrhythmias.
2. Quinidine,another drug which prolongs the QT interval,is associated with ventricular tachycardia in 0.5% to 8.8% of patients .There are structural similarities between acodazole and quinidine.
3. The ventricular arrhythmias which occur with quinidine and in the one patient affected by acodazole have a distinctive morphology.("torsades de pointes")
4. No antitumor activity was seen in the 38 patients who completed four or more weeks of therapy with acodazole.
5. The potential for life-threatening arrhythmias must be recognized as the further development of acodazole is considered.

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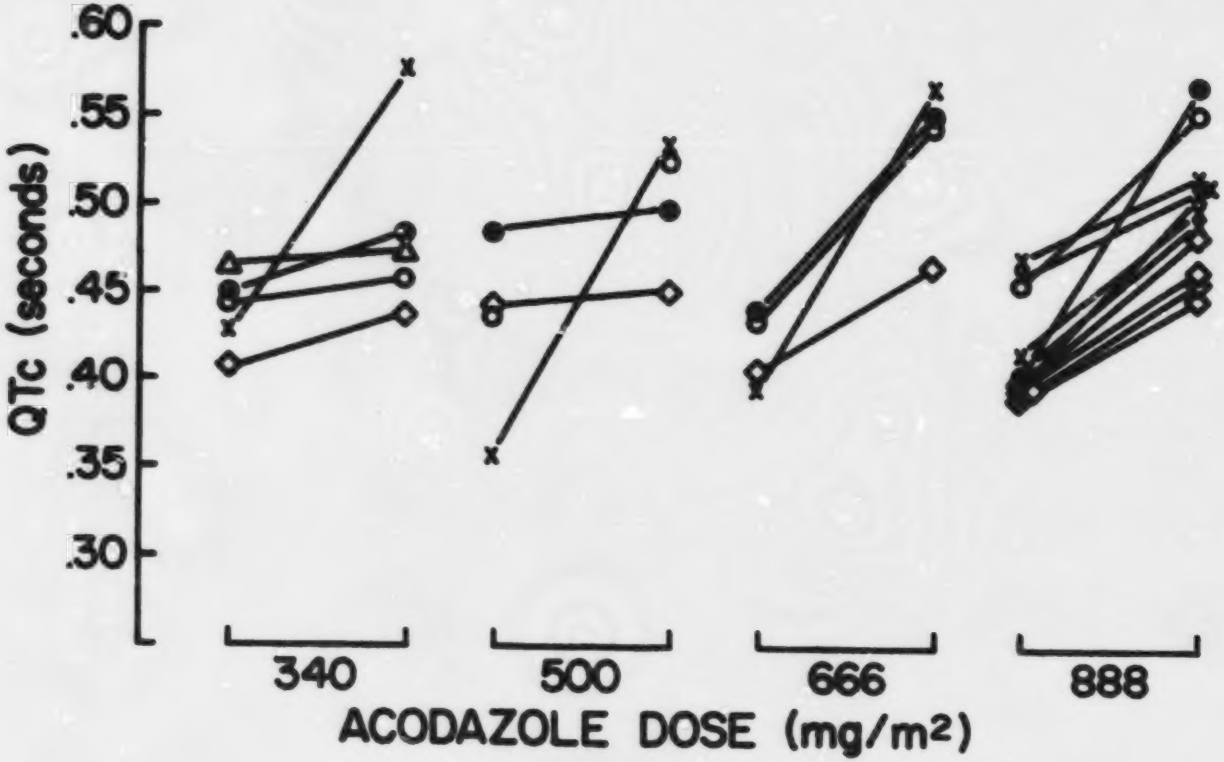
MAXIMUM CHANGE IN QTc (QT interval corrected for rate)



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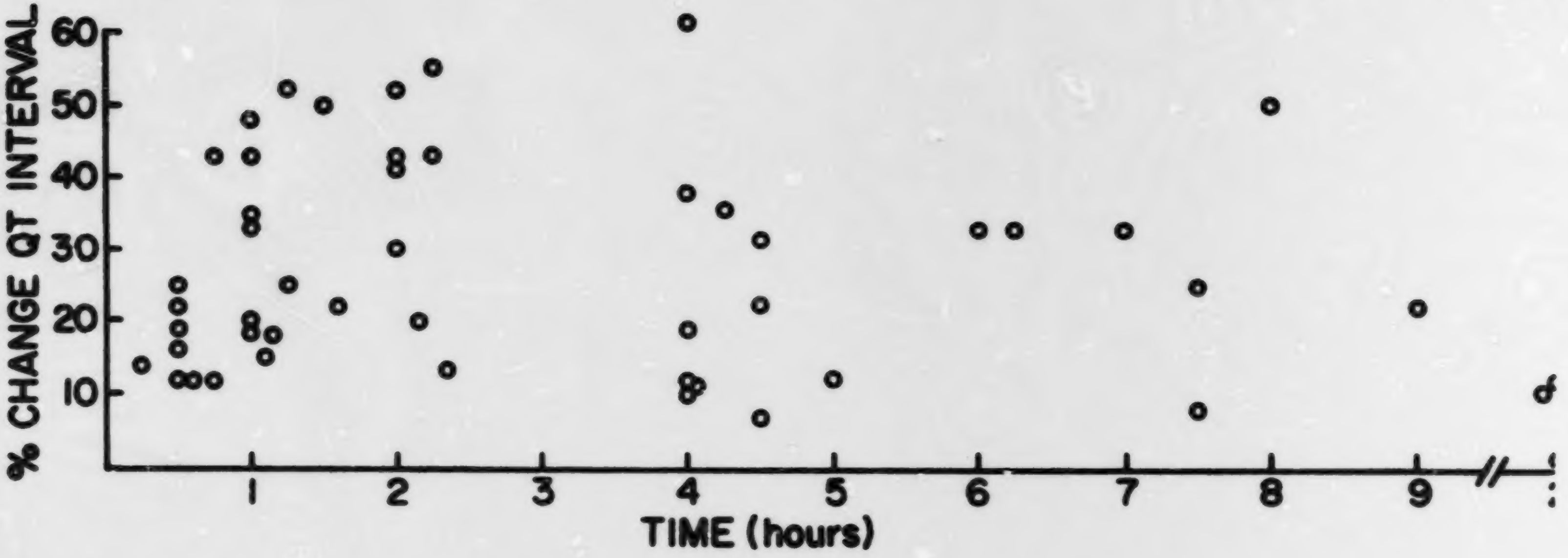
PERCENT CHANGE QT INTERVAL DURING AND FOLLOWING ACODAZOLE INFUSIO



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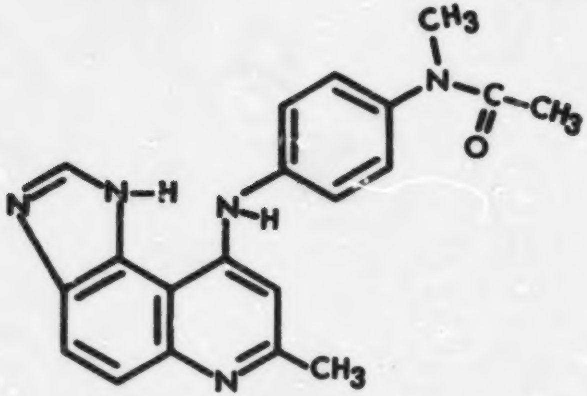
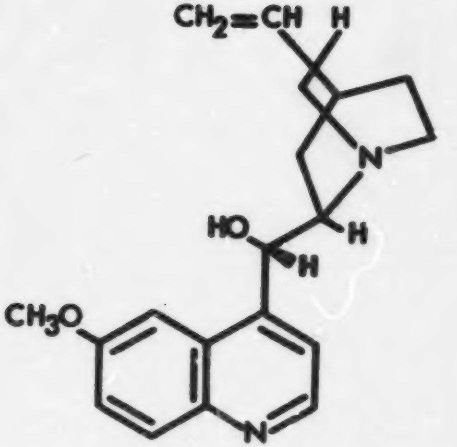

CH3
N
C
CH3
O
N-H
N
N-H
N
CH3

ACODAZOLE

7-Methyl-9-arylamino
imidazoquinoline


CH2=CH
H
N
HO
H
H
CH3O
N

QUINIDINE

6-Methoxy-α-(5-vinyl-2-quinuclidinyl)-
4-quinolinemethanol

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ACODAZOLE PHARMACOKINETIC PARAMETERS DERIVED FROM A 3-COMPARTMENT MODEL

Patient	Dose	Peak C_p	AUC_t	$t_{1/2}\alpha$	$t_{1/2}\beta$	$t_{1/2}\gamma$	MRT	Cl_t	Vc	Vd_{ss}	Vd_γ	Urinary recovery
	(mg/M^2)	(mcg/ml)	(mg x hr/L)	(min)	(hr)	(hr)	(hr)	($ml/min/M^2$)	(L/M^2)	(L/M^2)	(L/M^2)	(% dose/ 72 hr)
GK	888	13.60	59.6	4.24	1.33	18.4	18.9	240	10.4	282	396	32.4
GR	888	9.54	52.8	5.64	1.76	16.4	17.9	280	21.4	301	397	28.6
BO	886	10.30	73.5	6.35	2.93	26.5	29.2	202	20.2	354	464	17.2
PT	888	11.00	66.2	5.58	2.62	18.7	19.2	224	18.6	258	363	30.8
DM	666	6.85	25.5	8.22	2.37	16.9	15.6	434	29.4	406	635	23.6
ZB	666	7.84	49.1	11.90	3.29	24.6	26.3	226	32.8	356	481	10.6
EG	666	8.24	51.9	12.20	2.17	21.7	23.6	214	40.3	303	401	32.0
GA	500	6.65	35.2	6.08	2.78	19.1	19.2	237	16.2	274	392	17.2[a]
SV	500	5.88	24.4	7.37	2.23	16.4	15.6	342	23.2	320	486	31.4
WD	500	11.00	47.9	4.35	2.00	33.0	32.0	173	6.6	333	501	27.0[a]
CR	340	4.81	23.6	8.99	2.20	23.0	22.2	240	22.1	320	475	38.0[a]
Mean	—	—	—	6.55[b]	2.21[b]	20.7[b]	20.2[b]	256	21.9	319	454	25.8[c]
±S.D.								±73.5	±9.6	±42.3	±76.7	±8.1

[a] 24-Hr collection only.

[b] Harmonic mean.

[c] Mean of 72-hr values (n = 8).

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Amonafide
T84-0491
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BACKGROUND

- Benzisoquinolinedione is a novel synthetic derivative of 3-nitro-1,8 naphthalic acid.
- It inhibits DNA synthesis and functions as a DNA intercalator.
- It is active against leukemias L1210 and P388, B-16 melanoma and murine sarcoma M5076. Best ILS (110-144%) occurred with multiple dosing, (Days 1-5; 1-9; 1,5,9).
- The LD10 in mouse was 180 mg/m^2 x 1 or 75 mg/m^2/d x 5; 1-2 x MELD10 in dog was nontoxic.

METHODOLOGY

Patients - Adults with metastatic solid tumors, PS 0-3, failed standard therapy, normal hepatic and renal function and normal blood counts.

Drug Schedule - Starting dose, 1/10 LD10 = 10 mg/m^2/d x 5 days q 3 wks. Dose escalated 50%-100% to grade 2 toxicity. Drug mixed in normal saline, infused over 30-60 minutes.

Monitoring - Blood counts twice/weekly, SMA, urinalysis q 1-2 weekly, tumor measurements q 3-4 weeks.

Pharmacokinetics in selected patients.

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PHASE I STUDY OF BENZISOQUINOLINEDIONE (BIDA)

PATIENT CHARACTERISTICS

Number of Patients Entered:	28
Age, median (range):	51 (25-67)
Sex:	
Female	11 (39%)
Male	17 (61%)
Performance Status:	
0,1	19 (68%)
2,3	9 (32%)
Prior Therapy:	
Chemotherapy	13 (46%)
Radiation and Chemotherapy	15 (54%)
Prior Chemotherapy:	
Number of Regimens:	
1	6 (21%)
2	9 (32%)
3	13 (47%)

PHASE I STUDY OF BIDA

Tumor Types

Colorectal Carcinoma	8
Lung	8
Sarcoma	4
Melanoma	3
Breast	2
Head and Neck	1
Esophagus	1
Unknown 1^o	1

BIDA: DOSE ESCALATION SCHEME

Dose mg/m^2	No. of Patients	No. of Courses		
		Initial	Subsequent	Total
10	3	3	0	3
20	7	4	3	7
30	3	1	2	3
45	5	2	3	5
70	4	0	4	4
110	4	2	2	4
170	4	1	3	4
260	6	3	3	6
400	13	10	11	21
500	6	4	3	7
625	1	0	2	2

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PHASE I STUDY OF BIDA

HEMATOLOGIC EFFECTS

Dose Level mg/mm^{2}x5	No. Evaluable Courses	Lowest Median Counts X 10^3/mm^3	
		Absolute Granulocyte Count (Day) (Range)	Platelet (Day) (Range)
10-170	30	4.0 (12) (2.2-10.6)	309 (11) (197-572)
260	6	3.8 (15) (0.8-15.7)	265 (13) (71-560)
400	21	0.8 (15) (0-4.4)	130 (15) (5-500)
500	7	0.3 (15) (0-2.3)	103 (15) (6-239)
625	2	1.5 (15) (1.3-1.7)	197 (13) (191-203)

NON-HEMATOLOGIC TOXICITY OF BIDA

	Dose mg/m^2/day x 5 days						
	10 – 170	260	320	400	500	625	Total
Evaluable Courses	30	6	4	21	7	2	70
Nausea/Vomiting	1	1	1	16	5	2	26
Dizziness	-	1	-	10	4	1	16
Diaphoresis	-	1	-	7	2	-	10
Skin Rash	-	-	-	4	1	-	5
Tinnitis	-	-	1	3	1	-	5
Phlebitis	1	-	-	2	-	-	3

CLINICAL TOXICITY OF BIDA

Number of Evaluable Patients	27
Nausea/Vomiting	19 (70%)
Dizziness/Vertigo	12 (44%)
Diaphoresis	9 (33%)
Ear Fullness/Tinnitus	7
Skin Rash	5
Alopecia	4
Phlebitis	2
Dyspnea	1

ANTITUMOR EFFECTS OF BIDA

Number of Evaluable Patients	24
Complete Response	0
Partial Response	0
Minor Response	1 (Lung CA)
Stable Disease	4 (Lung-3,Esoph-1)
Progression	19

CONCLUSIONS

- The dose limiting toxicity of BIDA was myelosuppression. Rapid administration was associated with dizziness, diaphoresis, tinnitus, and vomiting.

- Plasma pharmacokinetics data were evaluated using a one-compartment model. The mean plasma t 1/2 was 3.0 hrs. An average of 10-40% of the parent drug was excreted in the urine within the course of 5-day treatment. There was wide patient-to-patient variation in renal drug excretion.

- The MTD was 400 $mg/m^2/d$ x 5 days q 3 weeks for good risk patients and 300 $mg/m^2/d$ x 5 for patients with limited bone marrow reserve.

FIGURE 1

Analytical Methodology

HPLC: J. Chromatography (Biomed. Applications)
345: 408-412, 1985

Thermospray LC/MS: Courtesy of Vestech, Inc.
Houston, Texas

FIGURE 2

Preclinical Pharmacology: Mechanism(s) of Drug Action

1. Amonafide is an intercalating agent.
2. Produces both single and double strand breaks
3. Cellular drug uptake is rapid and reaches a steady state at 30 min at 37° C. Drug egress is rapid.
4. Since drug egress occurs at a rate similar to repair of DNA lesions, prolonged drug exposure may be required for optimal antitumor activity.

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FIGURE 3 Plasma Pharmacokinetics of Nafidimide In The Dog

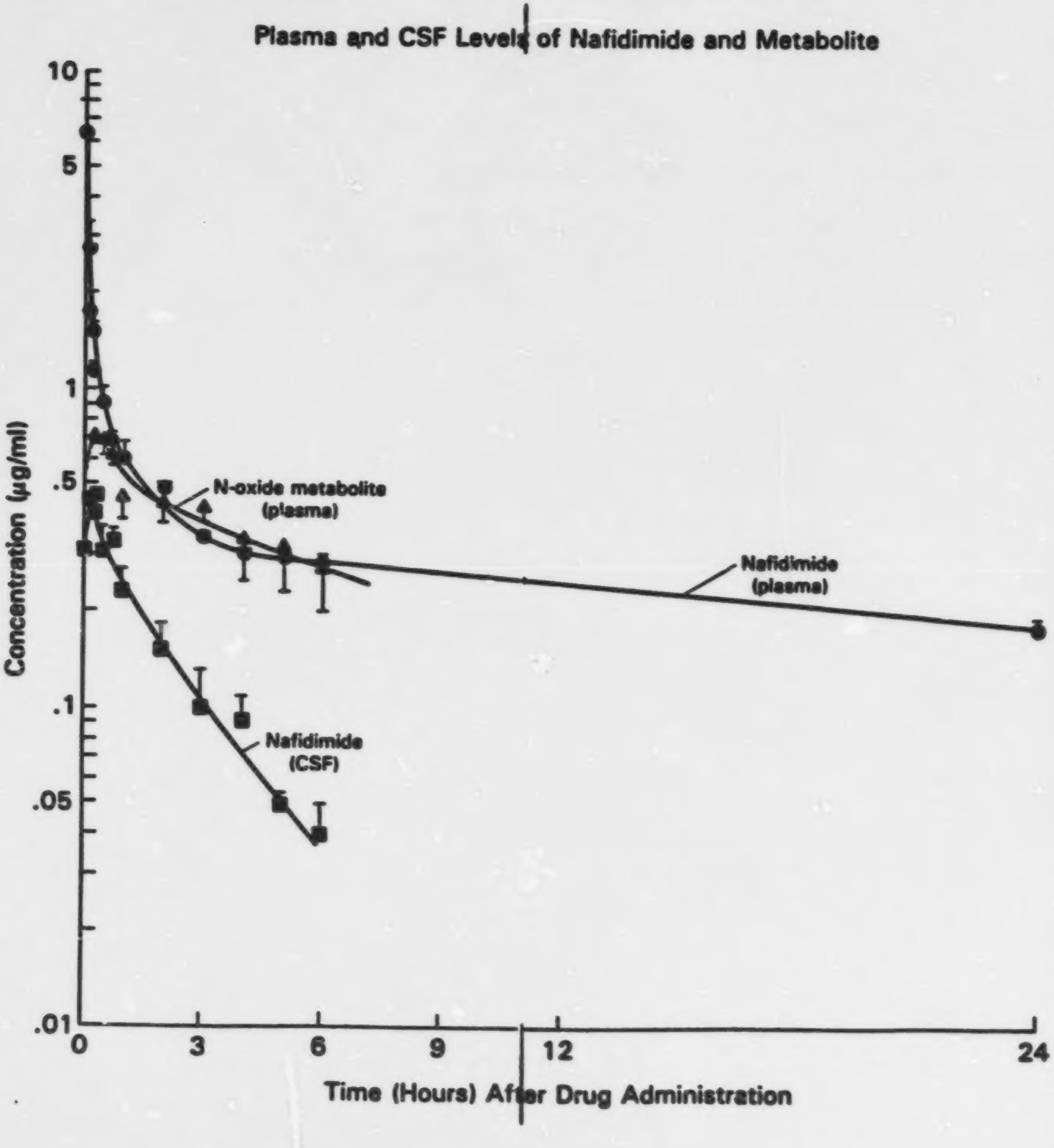

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FIGURE 4

Cumulative Urinary Excretion of Nafidimide and Metabolites

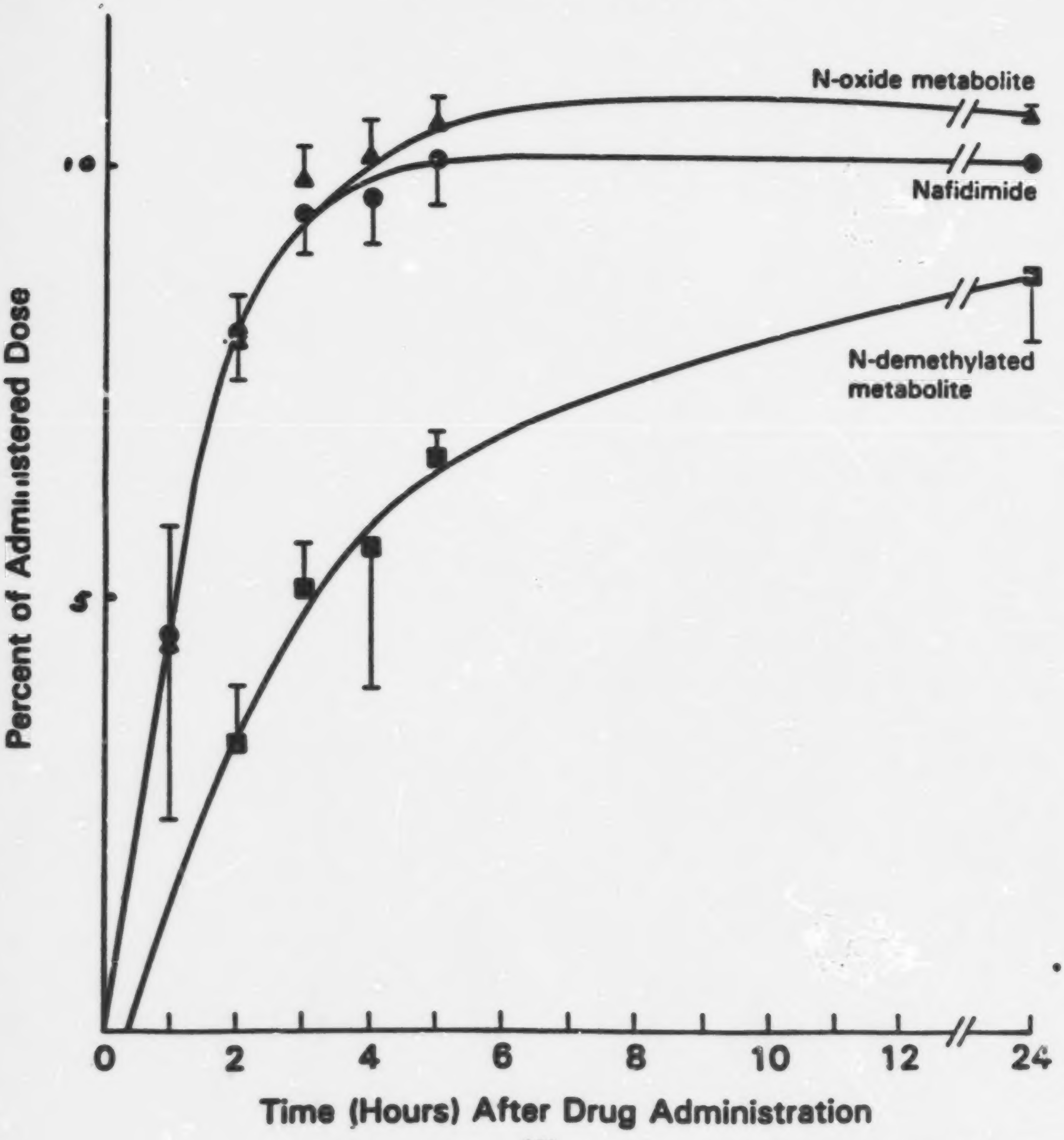


Figure 6

Tissue Concentrations of Nafidimide (6h) in the dog.

Data shown are those obtained 6 hours after drug administration.

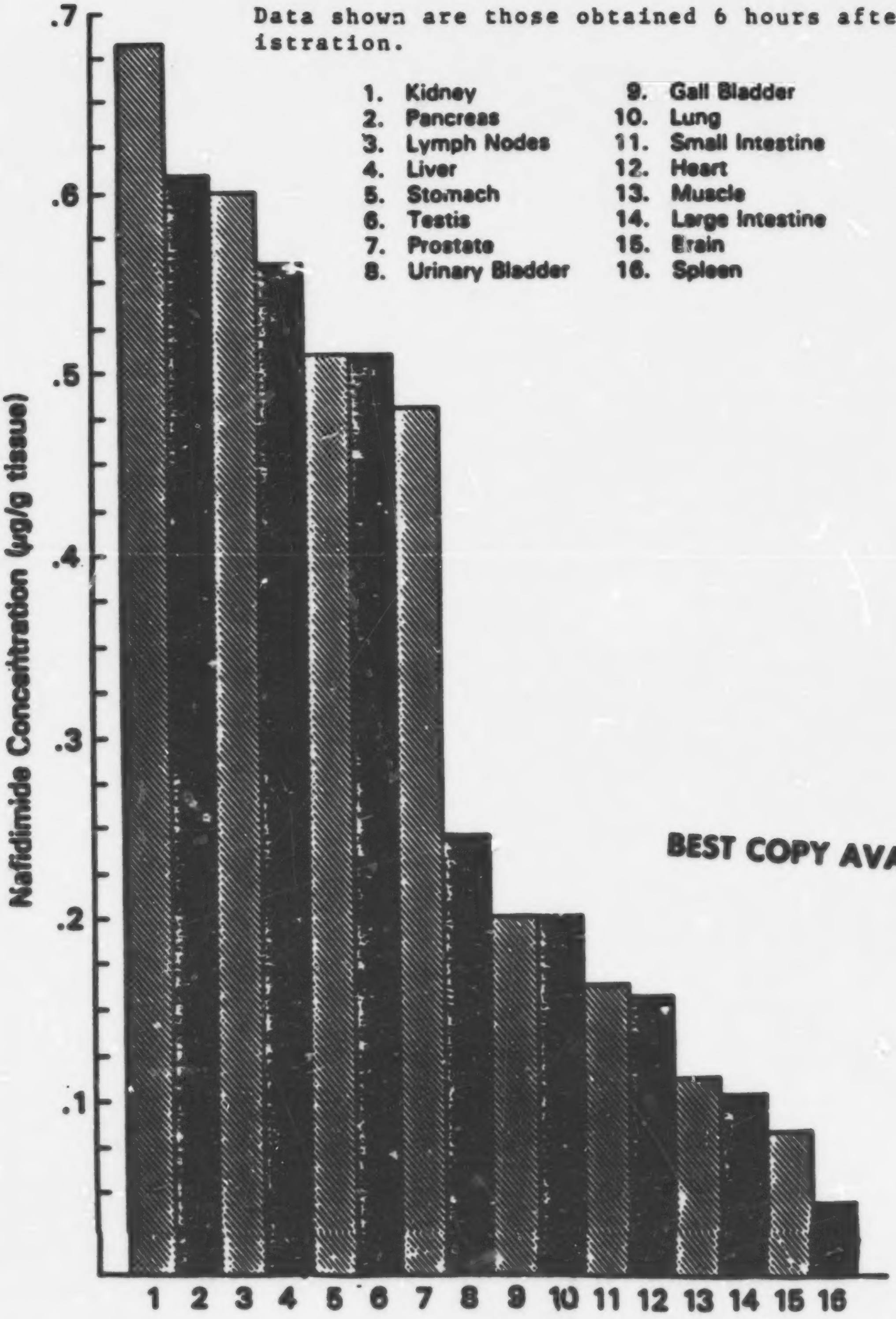
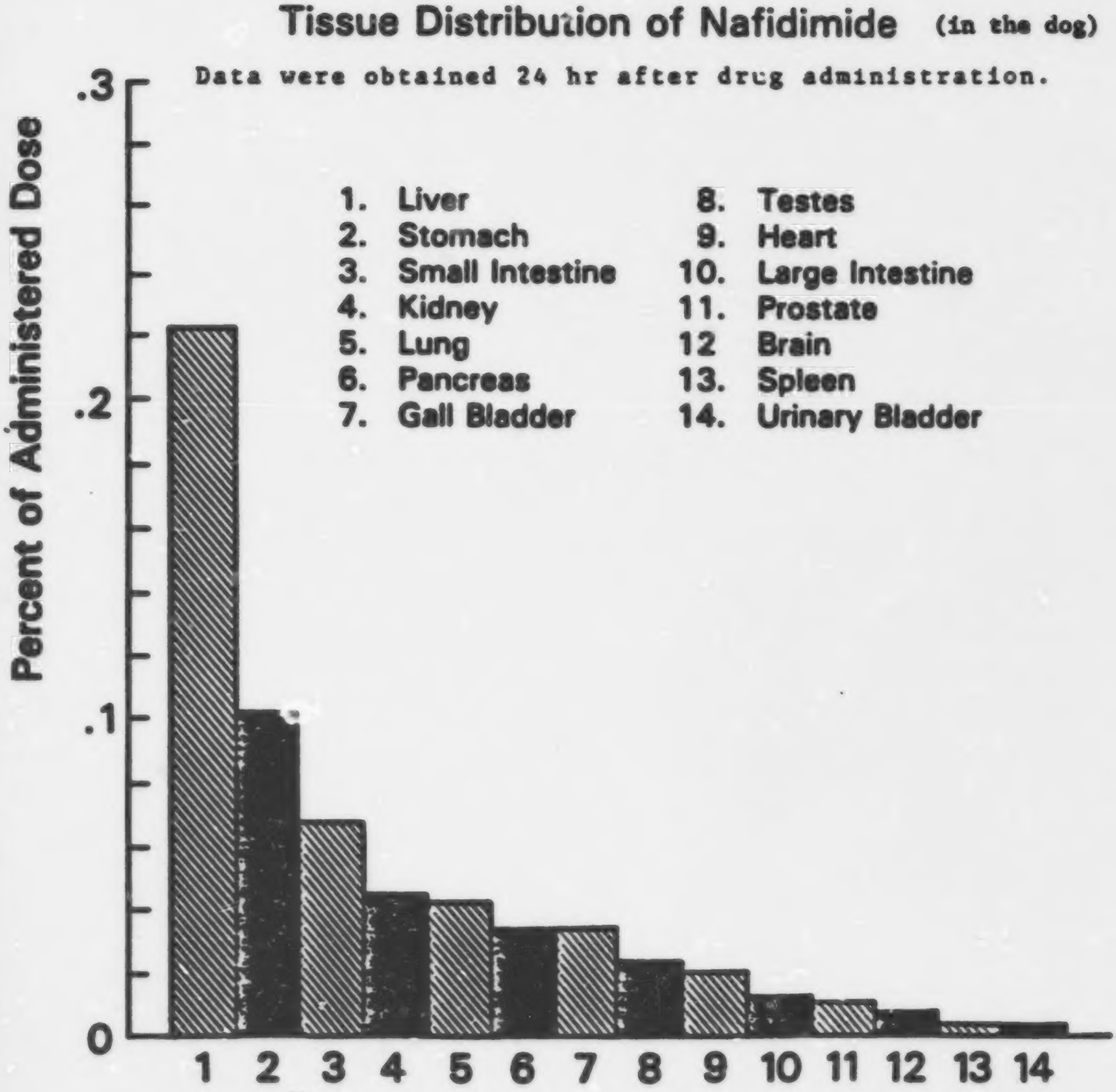


FIGURE 7



Tissue Distribution of Nafidimide (in the dog)

Data were obtained 24 hr after drug administration.

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Liquid Chromatography Separation of Nafidimide and Metabolites Prior To Analysis by Mass Spectrometry

FIGURE 8

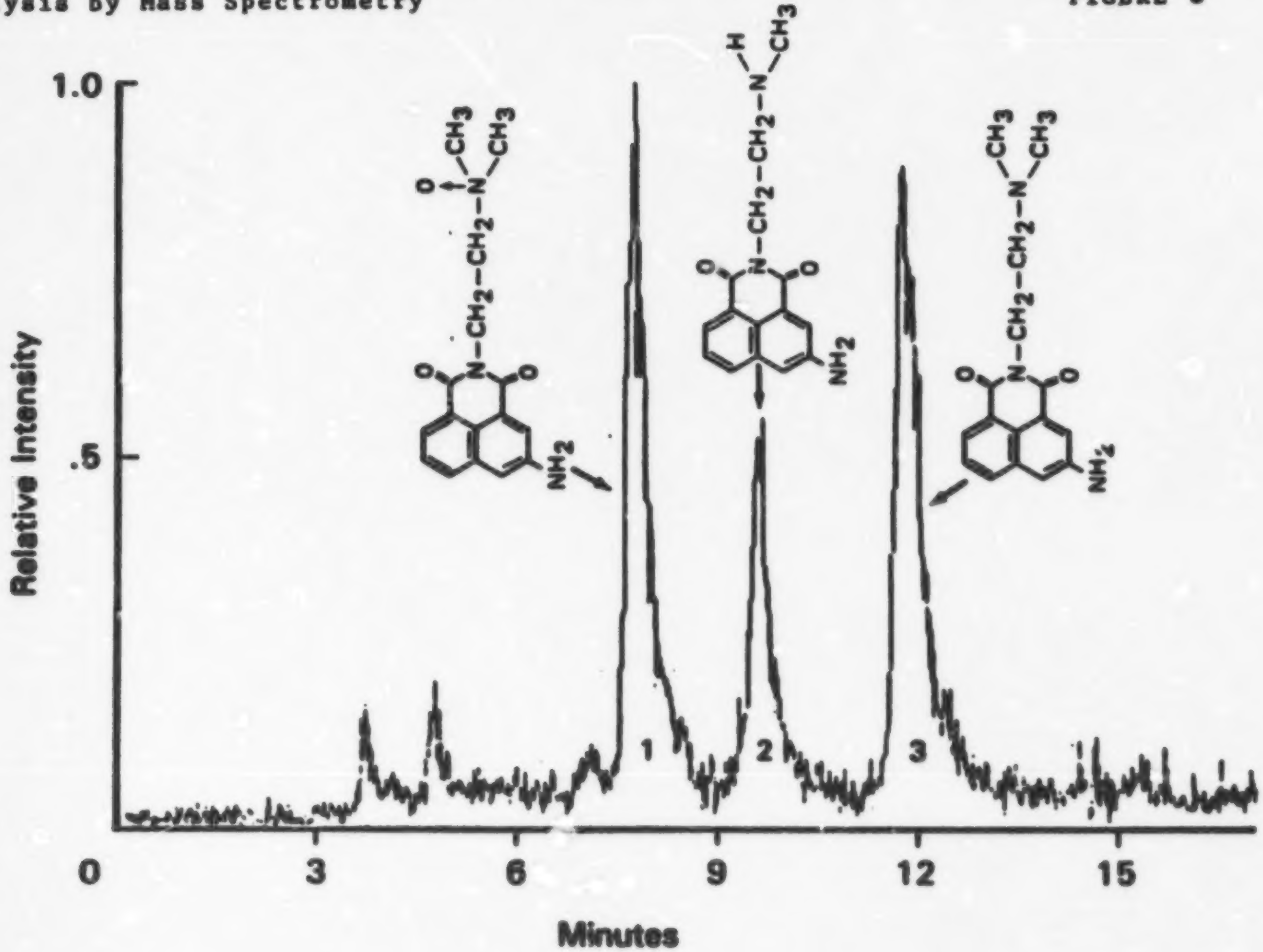

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FIGURE 9

Mass Spectrometry Data Obtained From Nafidimide Isolated From Dog Urine

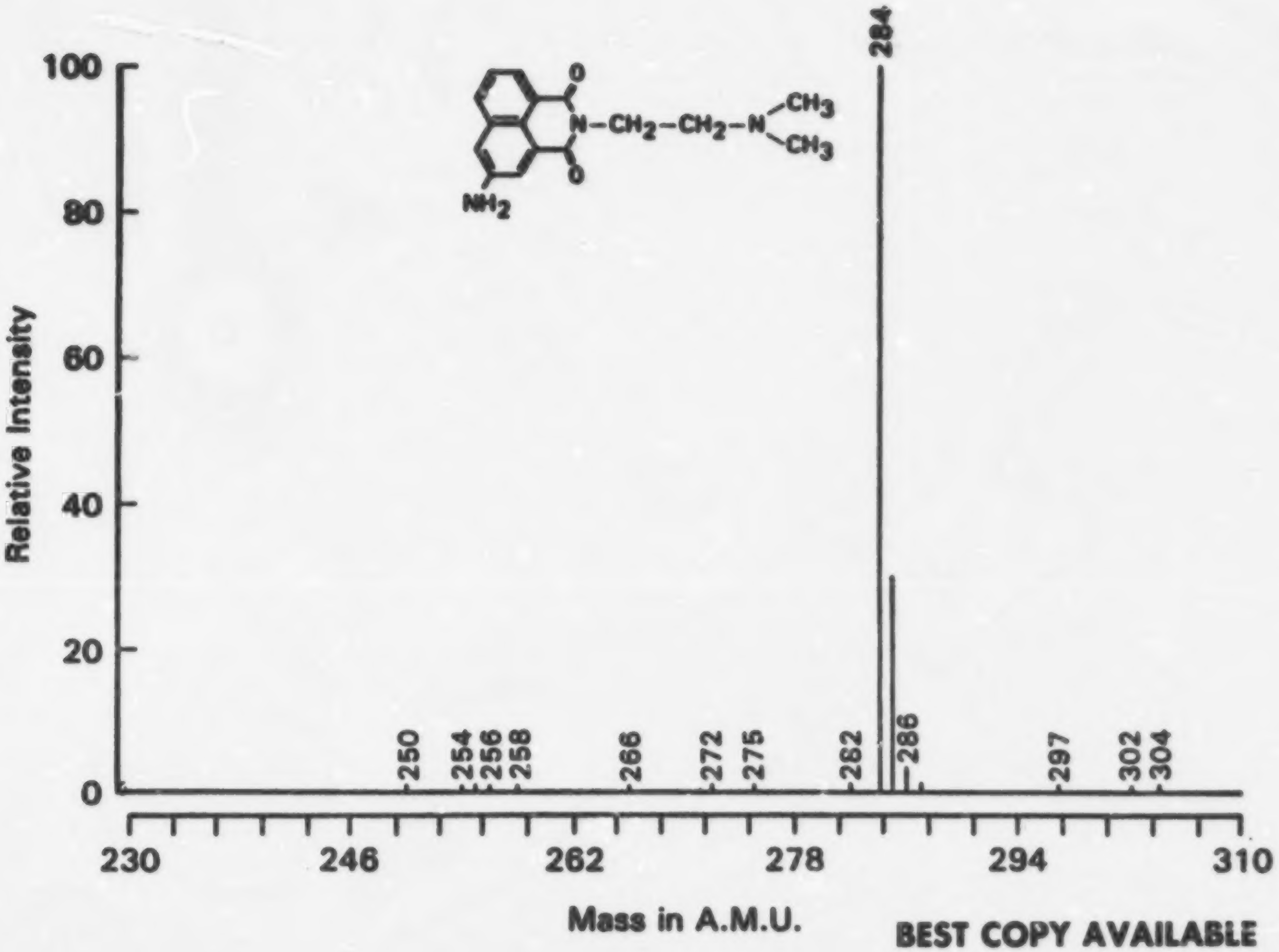

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FIGURE 10

Mass Spectrometry Data Of N-Demethylated Nafidimide Isolated From Dog Urine

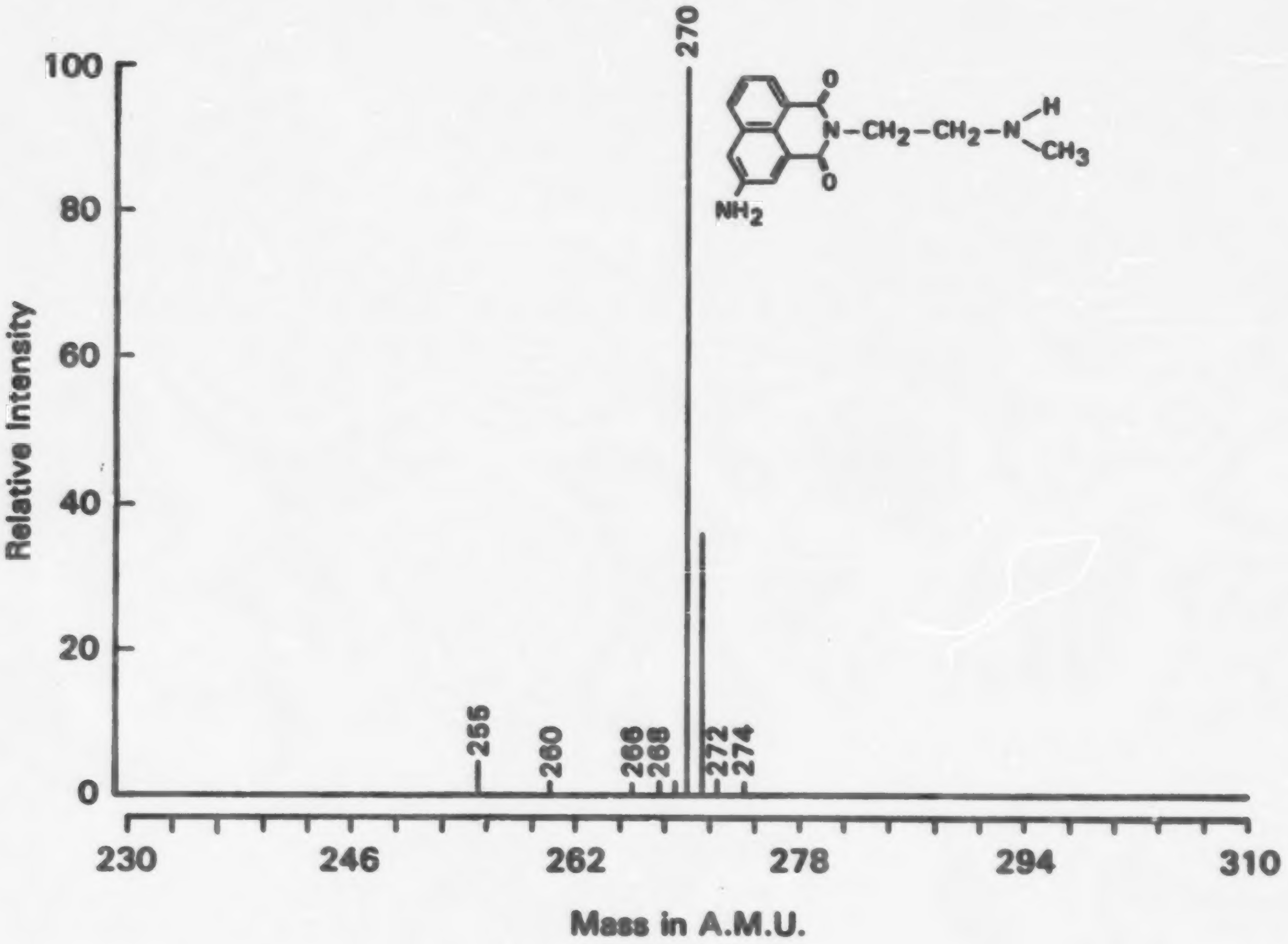

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FIGURE 11

Mass Spectrpmetry Data Of N-Oxide Nafidimide Isolated From Dog Urine

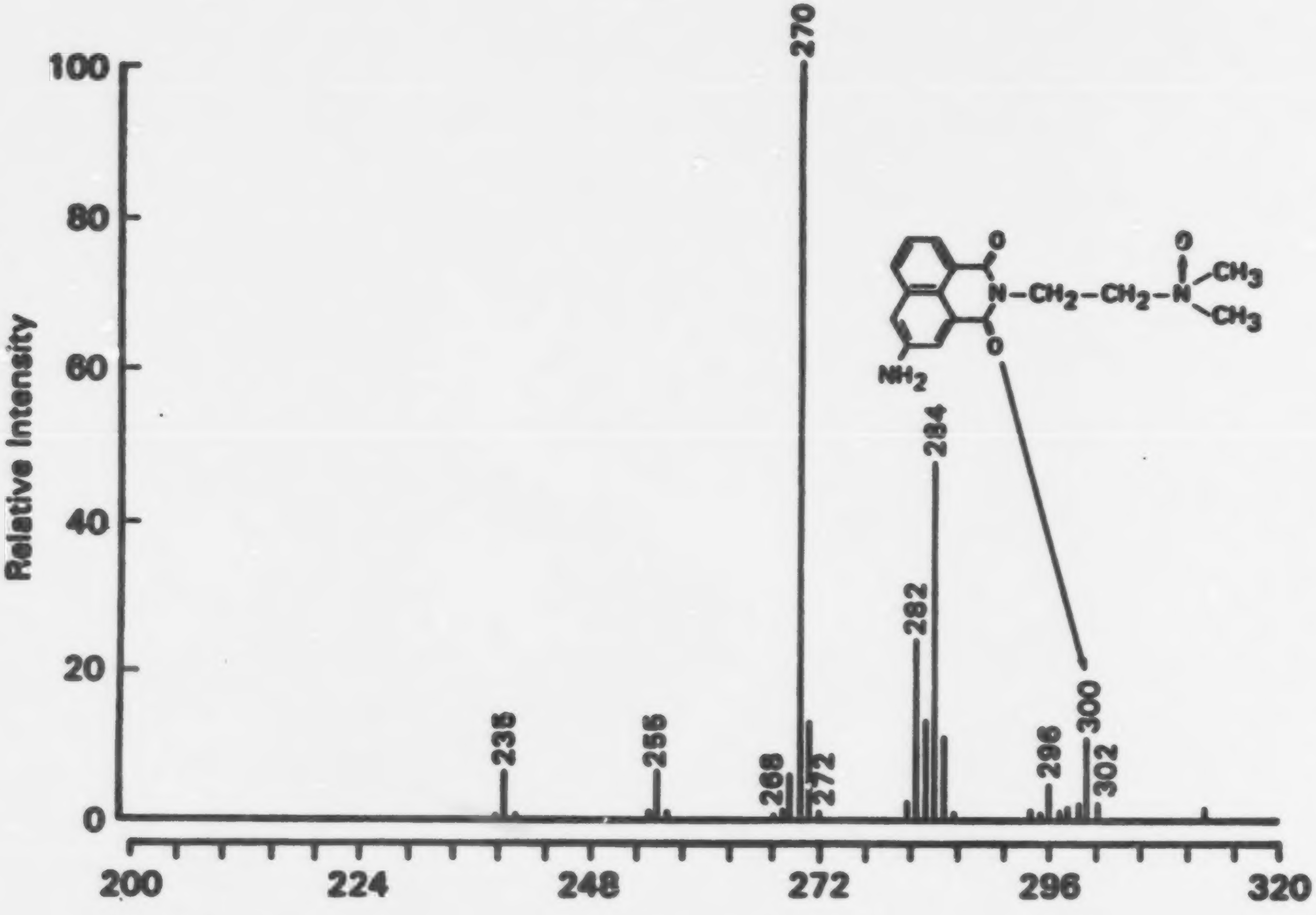

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FIGURE 12

Species-Dependent Metabolism of Amonafide

1. Major metabolites in the dog:

   N-desmethyl

   N-oxide

   N-acetylated species not observed

2. Major metabolites in humans:

   N-acetylated derivatives

   Few N-desmethylated species observed

FIGURE 13

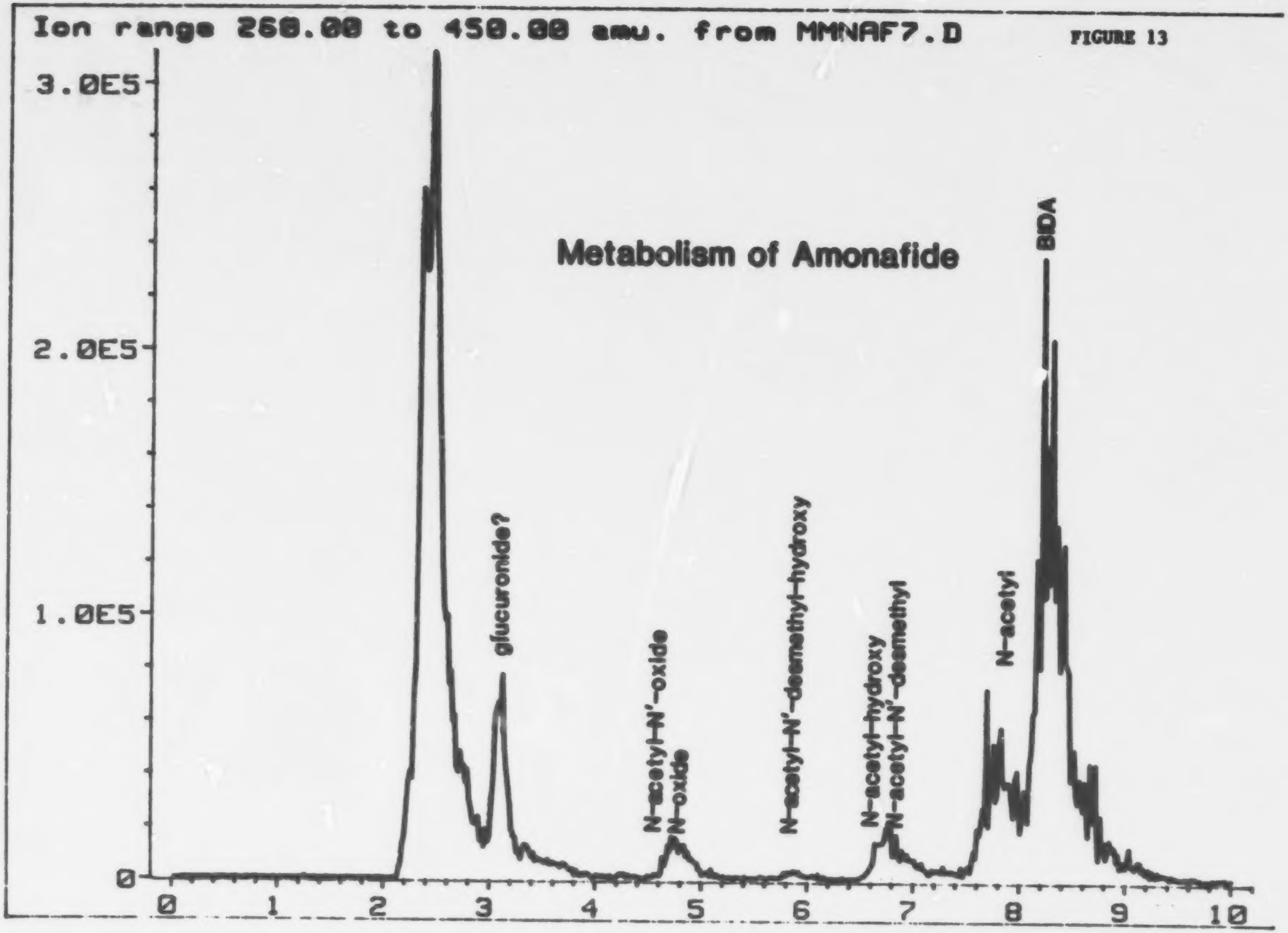

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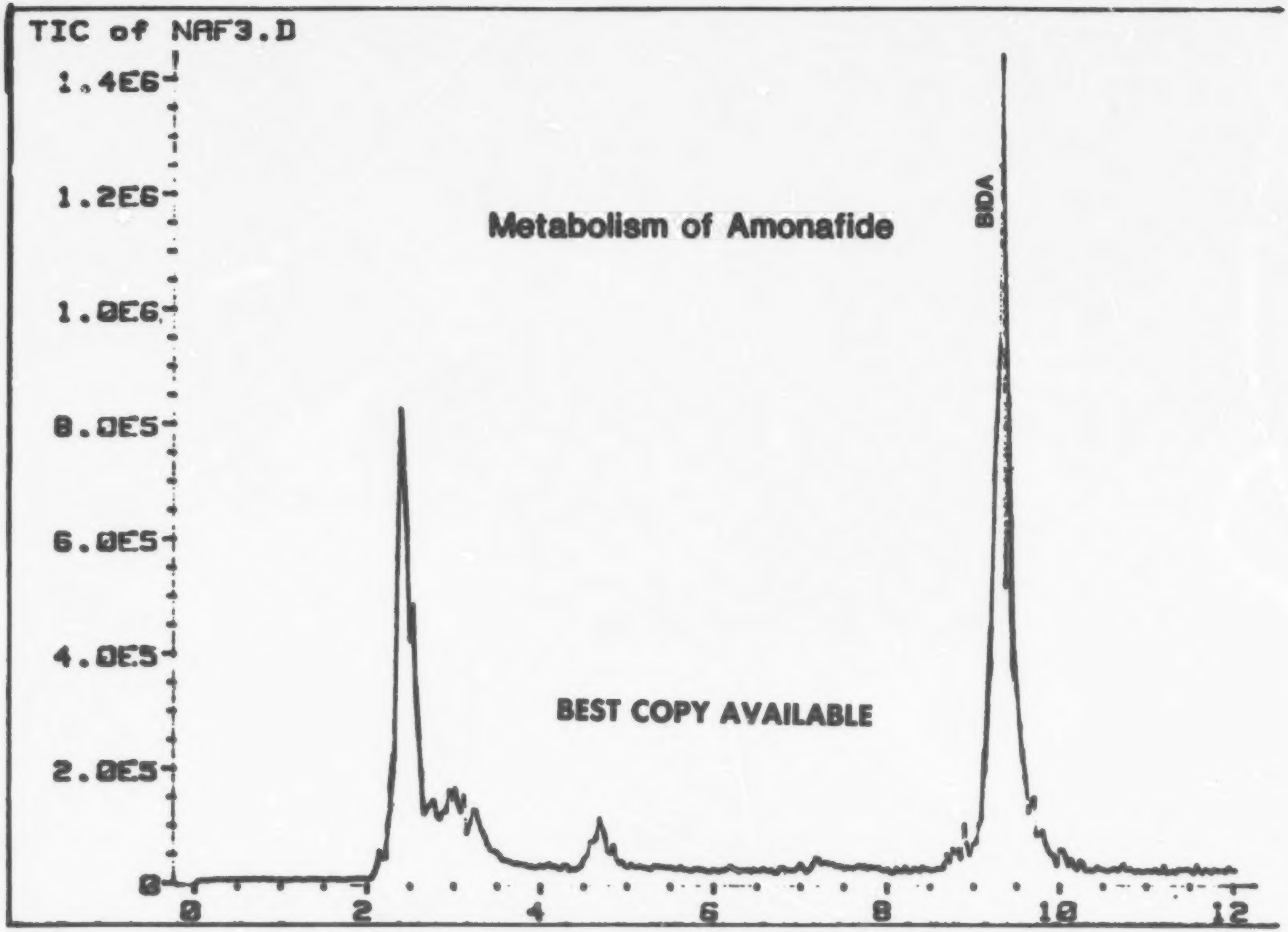
FIGURE 14



Metabolism of Amonafide

FIGURE 15

Clinical Pharmacology of Amonafide

1. Amonafide metabolism in the dog differs from that in humans.

2. Metabolism of amonafide in humans shows considerable patient-to-patient variation.

Clinical Amonafide Pharmacokinetic Parameters[a]

Patient	$V_d(\ell/m^2)$	$t_{1/2}(hr)$	AUC $\left(\frac{mg \cdot hr}{\ell}\right)$	$Cl_T\left(\frac{\ell}{m^2 \cdot hr}\right)$
1	42.1	1.9	26.6	15.1
2	141.2	3.8	15.5	25.6
3	159.4	4.5	16.4	24.3
4	109.9	2.4	13.0	30.7
5	107.0	2.0	10.9	36.6
6	124.2	2.6	11.9	33.1
7	374.8	7.1	11.0	36.6
Mean ± SD	151.2 ± 105.2	3.5 ± 1.9	15.0 ± 5.5	28.9 ± 7.8

[a]Dose = 400 mg/M²

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BENZISOQUINOLINEDIONE

METHODS

Two schedules were investigated, a single bolus schedule and a daily times five schedule. Infusions were delivered over 10 to 30 minutes initially. Each schedule started at 1/10 MELD 10 and escalated according to the modified Fibonacci search scheme.

BENZISOQUINOLINEDIONE

PATIENT CHARACTERISTICS

Number of Patients	51
Median Age (Range)	59 (29-77)
Performance Status	
0	5 patients
1	9
2	17
3	19
4	1
Prior Chemotherapy	44
Prior Radiotherapy	22

BENZISOQUINOLINEDIONE

PATIENT DIAGNOSES

Colorectal Carcinoma	12
Renal Carcinoma	9
Unknown Primary	4
Head and Neck Carcinoma	3
Ovarian Carcinoma	3
Salivary Gland Carcinoma	3
Small Cell Lung Carcinoma	3
Bladder Carcinoma	2
Breast Carcinoma	2
Sarcoma	2

*One patient each had cloacogenic, duodenal, esophageal, hepatocellular, non-small cell lung, pancreatic, and scrotal carcinomas. One patient had a neuroblastoma.

BENZISOQUINOLINEDIONE

HEMATOLOGIC TOXICITY-DAILY X 5

DOSE (MG/M^2/D)	NUMBER OF PATIENTS	NUMBER OF EVALUABLE COURSES	WBC NADIR* MEDIAN (RANGE)	PLATELET NADIR* MEDIAN (RANGE)
7.6	3	5	5.9(3.1-7.9)	254(125-304)
15.2	4	5	8.8(5.3-16.5)	291(233-427)
25.1	4	5	9.3(3.1-25.0)	215(73-407)
38.0	5	9	4.8(4.2-15.6)	304(183-595)
53.2	3	3	7.9(2.6-9.6)	175(91-509)
68.4	4	4	6.7(5.5-11.5)	350(127-467)
91.2	4	4	7.2(3.0-7.8)	185(145-409)
121.6	5	7	6.7(3.4-10.7)	318(80-378)
162.0	3	3	5.3(3.2-5.7)	213(114-246)
216.2	3	2	5.3(2.9-7.7)	283(224-342)

*WBC AND PLATELET COUNT x $10^3/\mu l$

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BENZISOQUINOLINEDIONE, DAILY x 5

NONHEMATOLOGIC TOXICITY

EPISODES OF TOXICITY (MEDIAN GRADE)

DOSE (MG/M^2/D)	NUMBER OF COURSES	GI	WARMTH/ FLUSHING	DIZZINESS
7.6	5	--	--	--
15.2	5	1(1)	--	--
25.1	5	--	--	--
38.0	9	2(1)	--	--
53.2	3	--	--	--
68.4	4	--	1(1)	--
91.2	4	2(2)	1(1)	1(2)
121.6	7	3(1)	3(1)	3(1)
*162.0	3	1(1)	2(1)	--
*216.2	2	1(1)	1(2)	--

*INFUSION LENGTHENED TO ONE HOUR

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BENZISOQUINOLINEDIONE

HEMATOLOGIC TOXICITY - SINGLE BOLUS

DOSE (NG/M²)	NUMBER OF PATIENTS	NUMBER OF EVALUABLE COURSES	WBC NADIR* MEDIAN (RANGE)	PLATELET NADIR* MEDIAN (RANGE)
18.4	3	3	3.7(3.6-7.6)	140(99-272)
36.8	4	6	9.1(5.3-13.3)	276(248-342)
60.7	5	7	8.6(3.1-12.8)	243(123-404)
92.0	7	8	5.0(4.0-8.9)	268(196-487)
128.8	4	4	7.9(3.3-11.8)	396(108-484)
165.6	4	4	5.8(2.7-6.7)	207(73-436)
220.8	4	10	6.5(4.4-8.7)	338(127-509)
294.4	5	6	4.7(3.7-24.8)	151(48-312)
392.5	3	4	17.7(6.0-30.8)	433(217-501)
523.4	8	11	3.6(1.5-9.3)	199(47-457)
697.8	1	1	4.1	452

*WBC and Platelet count x $10^3/\mu l$

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BENZISOQUINOLINEDIONE, SINGLE BOLUS

NONHEMATOLOGIC TOXICITY

EPISODES OF TOXICITY (MEDIAN GRADE)

DOSE (MG/M^2)	NUMBER OF COURSES	GI	WARMTH FLUSHING	DIZZINESS	EARS RINGING	SHORTNESS OF BREATH
18.4	3	1(1)	--	--	--	--
36.8	6	--	--	--	--	--
60.7	7	2(1)	--	--	--	--
92.0	9	--		--	--	--
128.8	4	1(1)	--	--	--	--
165.6	4	--	2(1)	--	--	--
220.8	10	2(1)	3(2)	5(1)	1(1)	1(2)
294.4	6	4(2)	4(1)	--	--	--
392.5	4	3(1)	3(1)	1(1)	--	--
523.4	2	2(2)	2(2)	2(2)	1(2)	2(3)
*523.4	10	6(1)	2(1)	--	--	--
*697.8	1	--	1(1)	--	--	--

*DURATION OF INFUSION INCREASED TO ONE HOUR

BENZISOQUINOLINEDIONE

SUMMARY

Fifty-one patients have received 113 courses. On the single bolus schedule, severe toxicity was encountered in two patients at a dose of 523.4 MG/M^2 given over 20 minutes. The patients developed the acute onset of vomiting, moderate flushing, dizziness, and tinnitus, and shortness of breath. One patient was given diphenhydramine and epinephrine for wheezing. Subsequent patients were treated with a 60 minute infusion with improvement in toxicity. It appears that myelosuppression may be the dose limiting toxicity on the single bolus schedule. Of eleven courses given at 523.4 MG/M^2, which are evaluable for hematologic toxicity, three were associated with grade 1 leukopenia, two with grade 2, and one with grade 3.

On the daily x 5 schedule, doses up to 216.2 MG/M^2 have resulted in no significant toxicity.

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AMONAFIDE

Pharmacology Report

THE OHIO STATE UNIVERSITY

INTRODUCTION

Clinical pharmacokinetic studies have been conducted in conjuction with the phase I evaluation of amonafide (NSC 308847, nafidimide, BIDA). Our present report describes the plasma pharmacokinetics in 27 patients following a single intravenous dose administered as a short infusion and in 12 patients on a daily x 5 schedule in which the drug is given as a short infusion daily for 5 days. The urinary excretion of the parent drug is reported in 26 of the patients on the single schedule. In addition, a summary of our studies to characterize the metabolites of amonafide is presented.

METHODS

1. The analytical method employed for the quantitation of amonafide in plasma and urine is described in the Phase I Working Group Minutes of November, 1985 together with typical calibration curves. Tables 1 and 2 provide a summary of the method.

2. As the doses were escalated from 92.0 to 392.5 mg/m^2 on the single schedule, the duration of the infusion of the drug was increased from 10 to 20 minutes and finally to 30 minutes. At 523.4 mg/m^2, side effects including flushing, dizziness, wheezing, and shortness of breath occurred in two patients, which appeared to be related to the rate of drug administration. Accordingly, the duration of the infusion was extended to one hour.

3. Plasma concentration-time data for each patient study on the single schedule was fitted by the non-linear least squares computer program NONLIN to a three compartment open model with first-order elimination from the central compartment using the equations for a short intravenous infusion.

4. Plasma concentration-time data from the multiple dosing schedule were NONLIN fitted to the multiple dosing equations for the classical three compartment open model using the microscopic rate constants and the central compartment volume of distribution as the iterated parameters. Constants for this subroutine included the daily dose and the beginning/end times for each day's infusion period. This multiple dosing subroutine for fitting the fifth dose data takes into account drug levels resulting from the terminal phase carry-over (accumulation) of the prior doses.

5. Amonafide concentrations in the 24 hour pooled urine of patients on the single schedule were determined for three days following dose administration.

6. The isolation of amonafide and its basic metabolites and the procedure for isolation of the acidic metabolite from urine were summarized in the November, 1985 minutes.

RESULTS AND DISCUSSION

1. Amonafide was eliminated by a first-order process and plasma levels exhibited tri-exponential decay (Figs. 1-4). The plasma levels declined approximately 1000 fold during 72 hr after dosing. The curve in the plasma profiles drawn through the experimentally determined concentrations represent the computer fit to the data.

2. For 27 patients on the single dose schedule for the dose range 92.0-523.4 mg/m^2, the amonafide plasma profile is characterized by a short initial disposition phase ($t_{\frac{1}{2}\alpha}$ = 13.2 min) followed by a beta phase ($t_{\frac{1}{2}\beta}$ = 2.38 hr) and a prolonged terminal half-life ($t_{\frac{1}{2}\gamma}$ = 11.47 hr) (Table 3). In these patients, the terminal half-lives ranged from 5.2 to 17.6 hr.

3. The mean residence time of the drug in the body (Vd_{ss}/Cl_T) was 6.99 hr with a range of 3.9 to 13.2 hr (Table 3).

4. The mean total body plasma clearance for patients on the single schedule was 954 ± 254 ml/min/m^2 (mean ± S.D.) (Table 3). The individual Cl_T values ranged from 562 to 1786 ml/min/m^2.

5. The terminal phase generally started at 18 to 30 hr after dosing and corresponded to 32.46 ± 17.61% of the area under the plasma concentration-time curve (Table 4).

6. The mean central compartment volume of distribution, V_1, was approximately 251% of body weight indicating that the drug is highly bound to rapidly perfused tissues (Table 5).

7. The volume of distribution at steady state was extremely large (Vd_{ss} = 434 ± 148 L/m^2). The total body volume of distribution (Vd_{γ} = 1047 ± 430 L/m^2) was substantially greater than Vd_{ss} indicating a very high degree of tissue binding of the drug (Table 5).

8. The smallest of the microscopic rate constants was k_{31} (data not presented) suggesting that release of the drug from deep tissue binding sites is the rate determining process for elimination of the parent drug from the body.

9. Within the dose range 92.0 to 523.4 mg/m^2, there was no apparent dependence of total body clearance, pharmacokinetic rate constants or volume parameters on dose (Tables 3 and 5).

10. Amonafide plasma concentration-time profiles determined on days 5-7 for 12 patients on the multiple dose schedule (38.0-121.6 mg/m^2/day x 5) followed similar three compartment open model behavior: $t_{\frac{1}{2}\alpha}$ = 11.3 min; $t_{\frac{1}{2}\beta}$ = 2.40 hr; $t_{\frac{1}{2}\gamma}$ = 13.04 hr and MRT = 7.04 hr (Table 6).

11. The mean total body clearance in the multiple dose study was 1085 ± 255 ml/min/m^2 (Table 6).

12. None of the multiple dose mean pharmacokinetic parameters was significantly different from the corresponding mean parameters determined during the single dose schedule (Table 6).

13. The mean urinary excretion of the parent drug for 26 patients on the single schedule was 11.9% of the dose and the mean renal clearance was 111.7 ml/min/m^2 (Table 7). A wide range of renal clearance values was observed (7.7-294.9 ml/min/m^2). A significant number of the patients exhibited renal clearance which was greater than the glomerular filtration rate suggesting that there is active secretion of the parent drug. No evidence was obtained for the conversion of a metabolite to the parent drug in urine.

14. Amonafide is extensively metabolized. Amonafide and its basic metabolites were isolated from patient urine using an Amberlite XAD-2 resin column followed by preparative thin layer chromatography. Six metabolites were obtained (Table 8). The structures of these compounds have been derived from PMR and mass spectra (Table 8). Synthetic samples of 5 of the compounds were prepared (Figs. 5 and 6).

15. Four peaks corresponding to metabolites were observed in chromatograms of alkalinized plasma extracts (Fig. 7). Comparative and co-chromatography indicate that these metabolites are 5-(N-acetyl)-amonafide, noramonafide, 5-(N-acetyl,N-hydroxy)-amonafide, and 5-(N-acetyl)-amonafide N-oxide. The principal metabolites, based upon the fluorescence intensity of the chromatographic peaks, are thought to be 5-(N-acetyl)-amonafide and noramonafide.

16. Three urinary metabolites which have not been observed in plasma are amonafide N-oxide, 6-methoxyamonafide and 5-amino-1,3-dioxo-1H-benz(de)isoquinoline-2(3H)-acetic acid. The latter compound (Fig. 8) is the 5-amino analog of the aldose reductase inhibitor alrestatin and was isolated from patient urine as the methyl ester.

17. In general, amonafide is highly bound to tissue binding sites and the rate of release of the drug from these sites is slow. The drug is extensively metabolized and two major metabolic routes are N-acetylation and N-demethylation. A study of polymorphic acetylation in these patients is complicated by the further metabolism of 5-(N-acetyl)-amonafide. The pharmacokinetics of amonafide are not dose-dependent and no significant accumulation occurs during multiple dosing.

TABLE 1

EXTRACTION OF AMONAFIDE (BIDA) FROM PLASMA

PLASMA:	1.0 ml (low conc. curve) 0.5 ml (midrange conc. curve) 0.1 ml (high conc. curve)
ALKALINIZATION:	Saturated sodium borate, 3 ml
INTERNAL STANDARD IN EXTRACTION SOLVENT:	Acridone in ethyl acetate (3.4 ng/ml), 5 ml
EXTRACTION:	Reciprocating shaker, 10 min
CENTRIFUGATION:	3000 rpm, 10 min
EVAPORATION:	To dryness under N_2
RECONSTITUTION:	Mobile Phase, 100 µl

TABLE 2

HPLC ANALYSIS OF AMONAFIDE (BIDA) IN PLASMA

L.C.:	Isocratic
COLUMN:	Ultrasphere C_8, 5 µm (4.6 mm x 25 cm)
MOBILE PHASE:	Ammonium acetate (0.2 M)-Methanol (3:2, v/v)
FLOW RATE:	1.0 ml/min
FLUORESCENCE DETECTION:	249 nm, excitation 550 nm, cutoff
LOWEST CONC. ON STD. CURVE:	0.5 ng/ml

TABLE 3

AMONAFIDE MEAN TOTAL BODY CLEARANCE AND HARMONIC MEAN HALF-LIVES AND RESIDENCE TIMES AT EACH DOSE LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients n	Dose (mg/m^2)	$t_{\frac{1}{2}\alpha}$ (min)	$t_{\frac{1}{2}\beta}$ (hr)	$t_{\frac{1}{2}\gamma}$ (hr)	MRT (hr)	Cl_T (ml/min/m^2)
5	92.0	15.7	2.58	10.60	6.13	793
4	128.8	8.1	2.02	10.93	7.37	994
4	165.6	15.2	2.47	10.84	7.18	865
3	220.8	6.2	2.50	10.12	7.01	1055
5	294.4	20.7	3.12	13.37	7.20	1102
3	392.5	18.6	1.56	8.42	6.20	979
3	523.4	37.3	2.77	18.89	8.82	875
Mean (n=27)	-	13.2	2.38	11.47	6.99	954
S.D.	-	-	-	-	-	254
C.V.,%	-	-	-	-	-	26.6

TABLE 4

MEAN PERCENTAGE OF THE AREA UNDER THE AMONAFIDE PLASMA CONCENTRATION - TIME CURVE CORRESPONDING TO EACH OF THE TRI-EXPONENTIAL DECAY PHASES AT EACH DOSE LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients n	Dose (mg/m^2)	Alpha Phase %	Beta Phase %	Gamma Phase %
5	92.0	20.76	53.40	25.84
4	128.8	15.79	44.38	39.82
4	165.6	20.63	43.73	35.64
3	220.8	14.95	45.59	39.46
5	294.4	21.35	53.97	24.69
3	392.5	13.47	44.95	41.58
3	523.4	28.17	45.36	26.47
Mean (N=27)	-	19.49	48.05	32.46
S.D.	-	7.28	14.80	17.61
C.V.,%	-	37.4	30.8	54.3

TABLE 5

MEAN AMONAFIDE VOLUME PHARMACOKINETIC PARAMETERS AT EACH DOSE LEVEL FOLLOWING SINGLE IV ADMINISTRATION

Patients (n)	Dose (mg/m^2)	V_1 (L/m^2)	V_2 (L/m^2)	V_3 (L/m^2)	Vd_{ss} (L/m^2)	Vd_γ (L/m^2)
5	92.0	71.5	108.2	127.3	307.0	872.9
4	128.8	64.3	149.7	260.0	474.0	963.7
4	165.6	91.9	122.7	184.9	399.5	845.1
3	220.8	80.3	223.2	202.7	506.2	945.3
5	294.4	123.7	168.1	207.9	499.5	1311.4
3	392.5	83.2	113.8	193.0	389.9	860.8
3	523.4	139.6	126.9	233.9	500.4	1560.0
Mean (n=27)	-	93.0	143.1	197.9	433.9	1046.9
S.D.	-	37.0	58.2	126.5	148.0	429.6
C.V.,%	-	39.8	40.7	63.9	34.1	41.0

TABLE 6

COMPARISON OF THE MEAN AMONAFIDE PHARMACOKINETIC PARAMETERS DETERMINED FOR THE DAILY x 5 SCHEDULE WITH THE MEAN PARAMETERS OF THE SINGLE SCHEDULE

Parameter	Units	Daily x 5 Schedule (n=12)	Single Schedule (n=27)
Cl_T	ml/min/m^2	1005	964
$t_{1/2_\alpha}$	min	11.3	13.2
$t_{1/2_\beta}$	hr	2.40	2.38
$t_{1/2_\gamma}$	hr	13.04	11.47
MRT	hr	7.04	6.99
Alpha Phase	% of AUC	16.78	19.49
Beta Phase	% of AUC	54.17	48.05
Gamma Phase	% of AUC	29.05	32.46
V_1	L/m^2	88.9	93.0
V_2	L/m^2	159.5	143.1
V_3	L/m^2	227.3	197.9
Vd_{ss}	L/m^2	475.7	433.9
Vd_γ	L/m^2	1234.7	1046.9

TABLE 7

AMONAFIDE MEAN URINARY EXCRETION IN PATIENTS FOLLOWING SINGLE I.V. ADMINISTRATION

Dose (mg/m^2)	Patients (n)	Aex (days 1-3) (% of Dose)	Cl$_R$ (ml/min/m^2)
92.0	5	15.6	122.4
128.8	4	6.8	65.7
165.6	3	5.1	51.6
220.8	3	8.7	95.4
294.4	5	19.3	193.7
392.5	3	12.6	121.4
523.4	3	9.8	85.0
Mean	26	11.9	111.7
Range	26	0.8-30.9	7.7-294.9

TABLE 8

AMONAFIDE METABOLITES ISOLATED FROM URINE USING XAD-2 RESIN FOLLOWED BY PREPARATIVE TLC

METABOLITE	TLC (R_f)	SYNTHETIC COMPOUND PREPARED	500 mHz PMR	M.S.
noramonafide	0.14	x	x	CI,EI
5-(N-acetyl)-amonafide N-oxide	0.20	x	x	CI
amonafide N-oxide	0.24	x	x	Ci
5-(N-acetyl,N-hydroxy)-amonafide	0.27	x	a	CI
amonafide	0.42		x	CI,EI
5-(N-acetyl)-amonafide	0.42	x	b	CI,EI
6-methoxyamonafide	0.71		x	c

[a] PMR of metabolite. [b] PMR of metabolite with amonafide.
[c] Decomposed in source.

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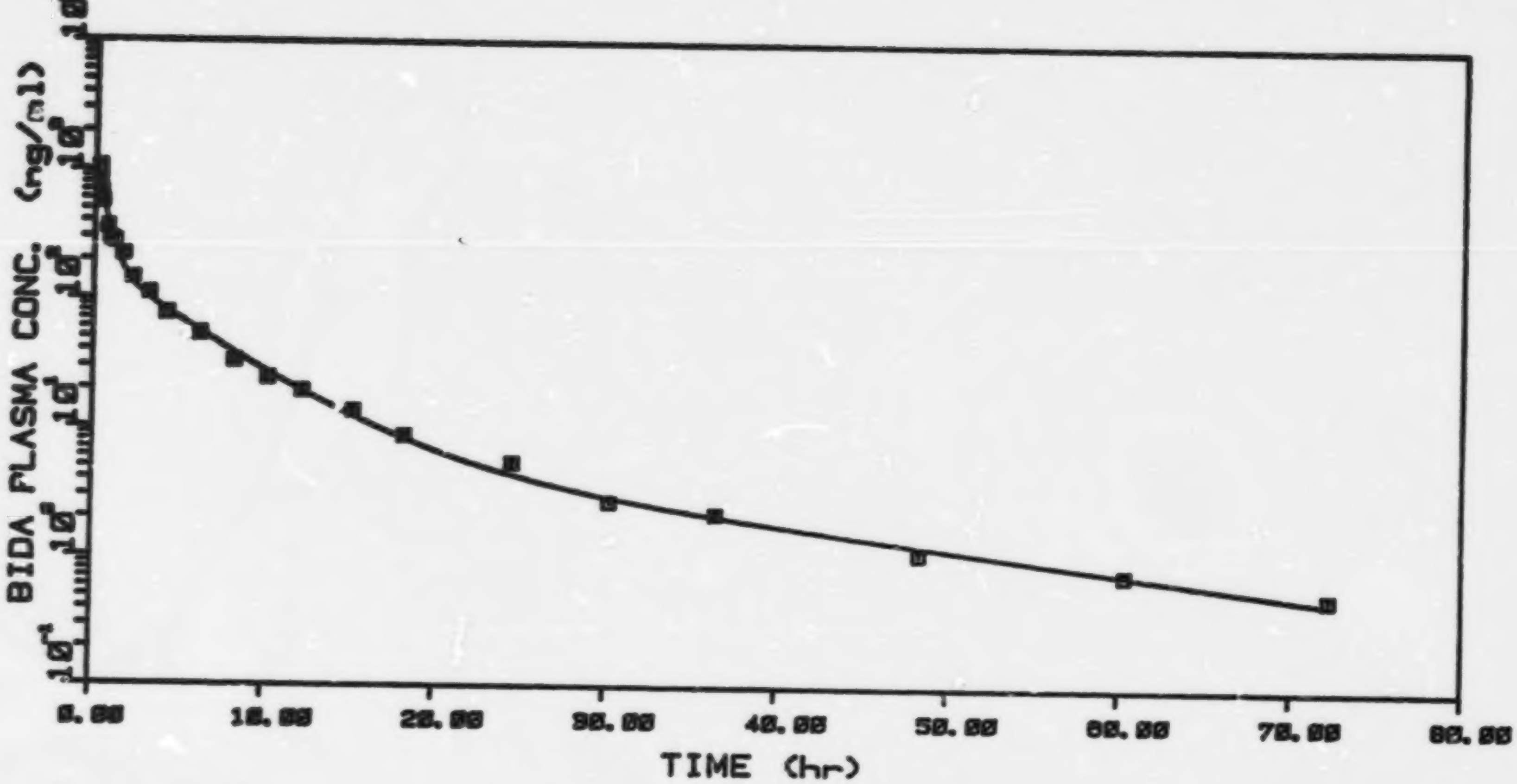

PATIENT C. S.
DOSE = 92.0 MG/METER SQ. BIDA
HALF-LIFE = 17.44 HOURS

Fig. 1

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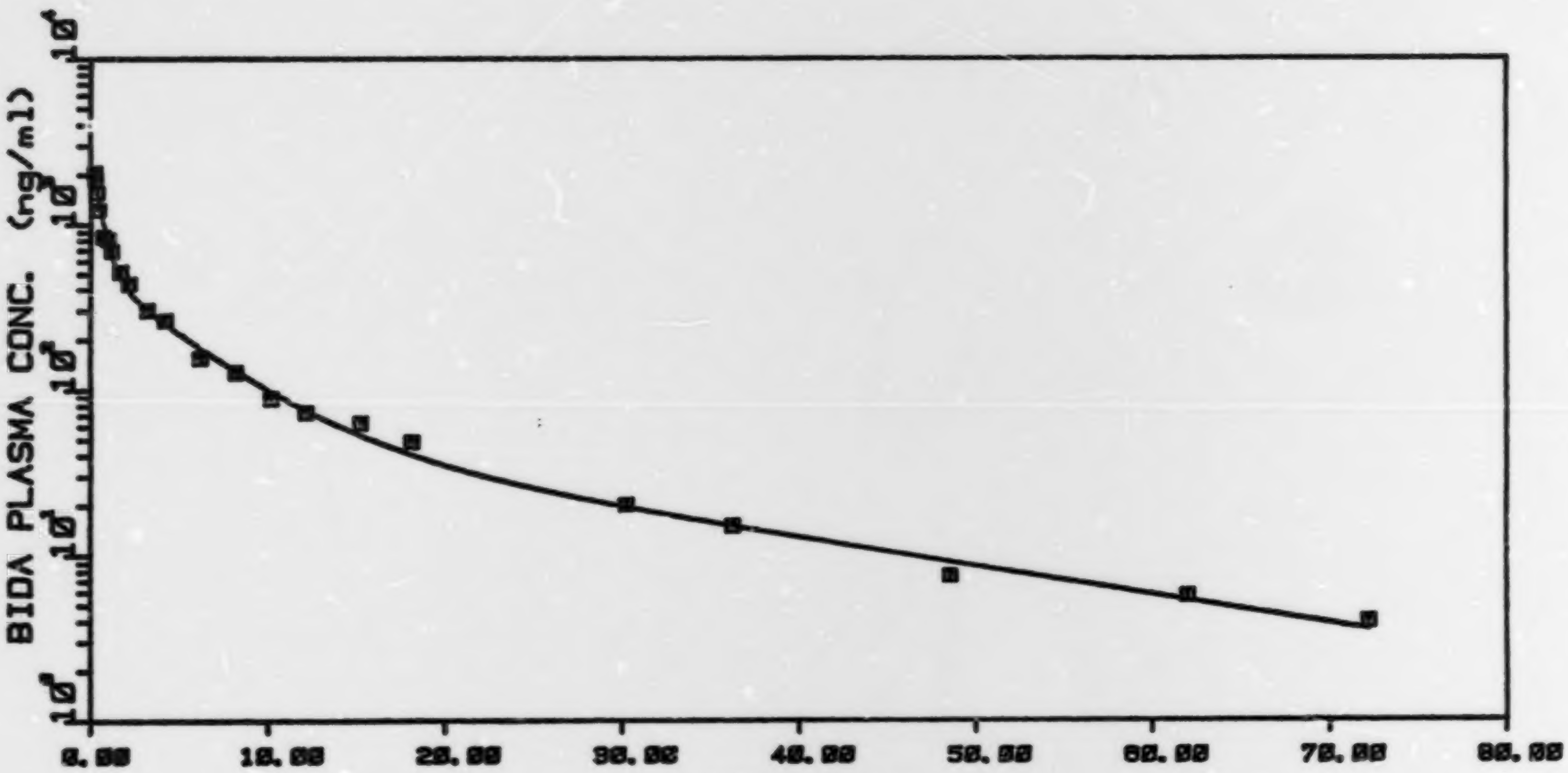

PATIENT W. C.
DOSE = 165.6 MG/METER SQ. BIDA
HALF-LIFE = 17.59 HOURS

Fig. 2

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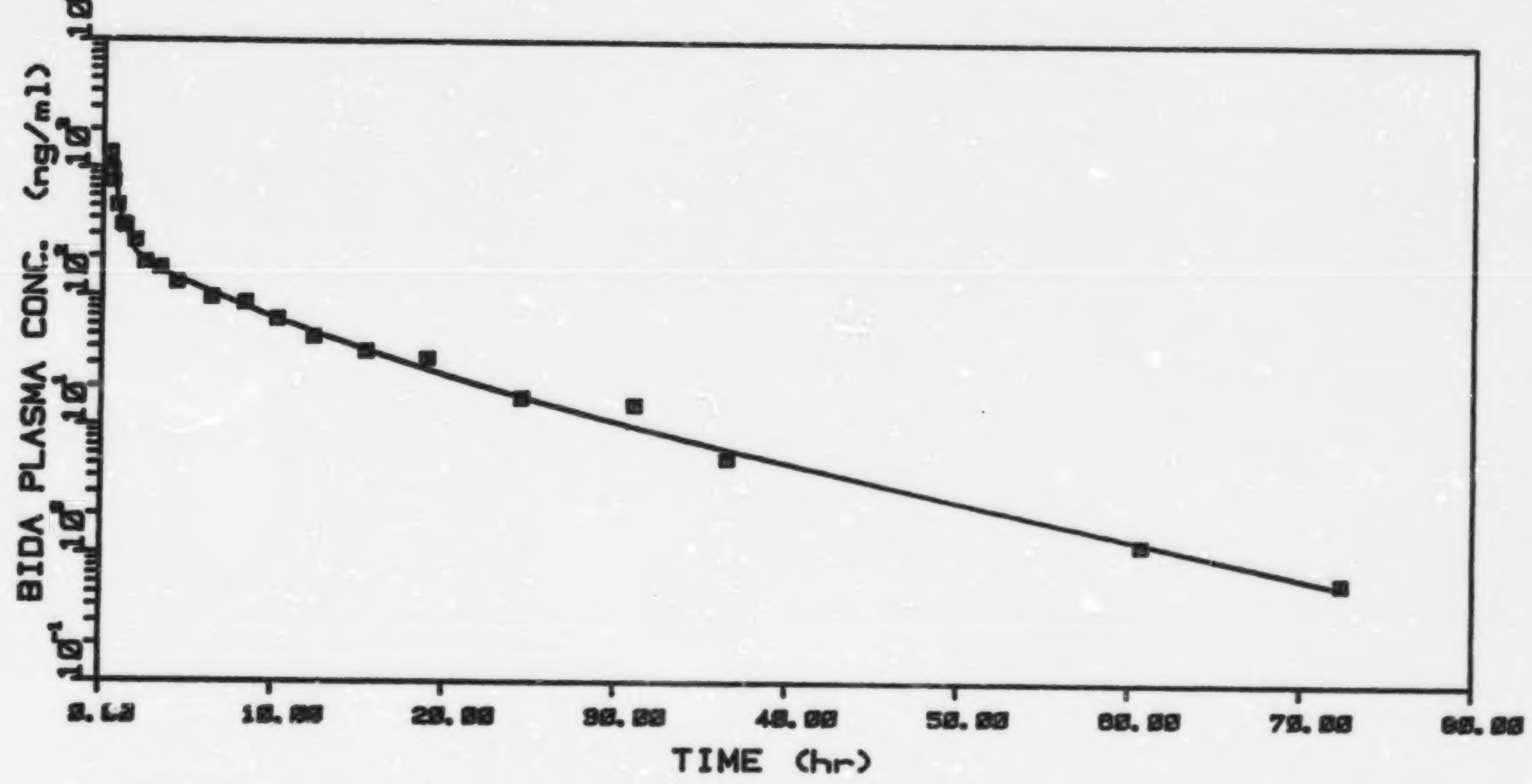

PATIENT J. B.
DOSE = 220.8 MG/METER SQ. BIDA
HALF-LIFE = 10.39 HOURS

Fig. 3

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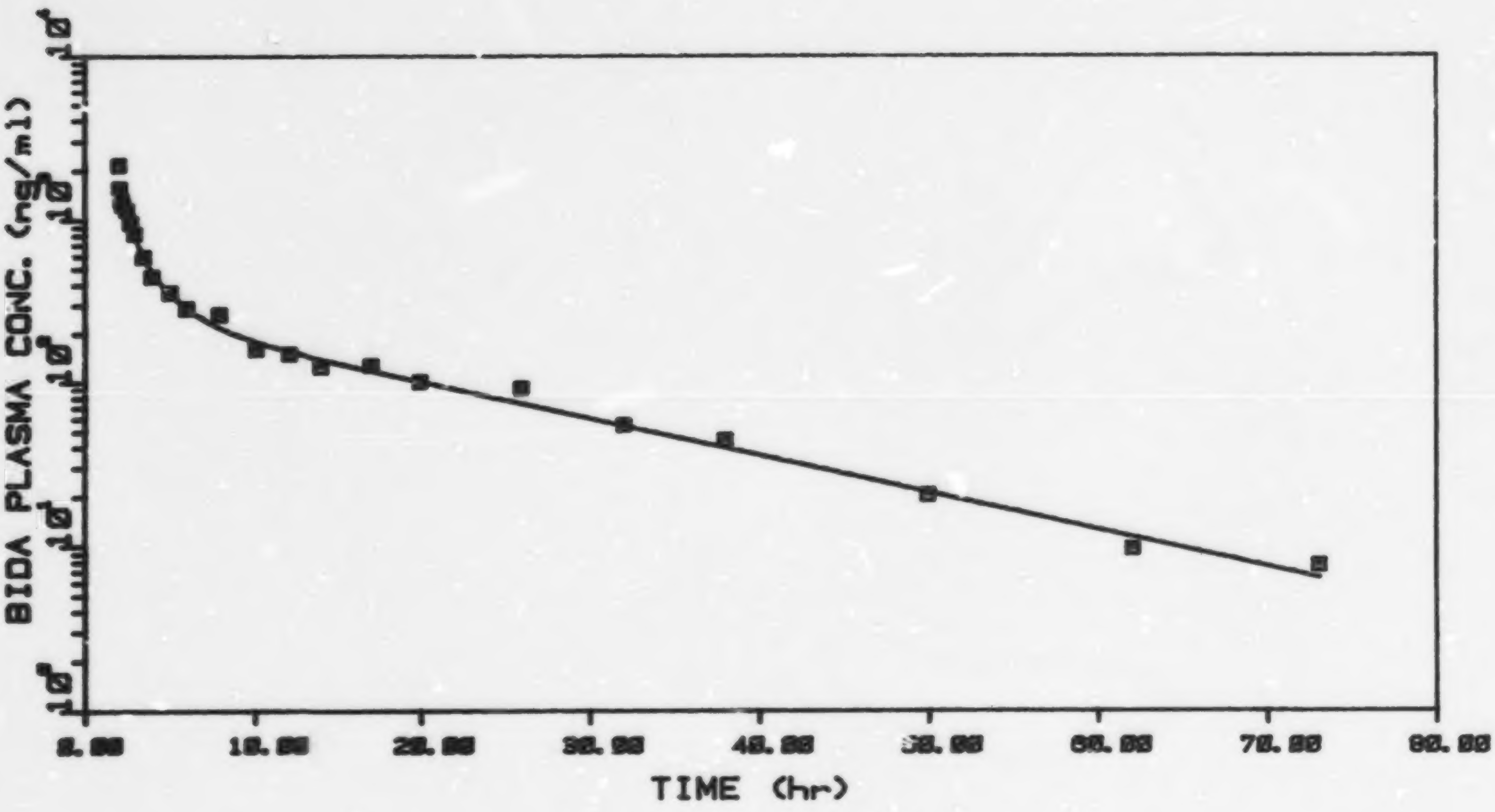

PATIENT A. T.
DOSE = 523.4 MG/METER SQ. BIDA
HALF-LIFE = 13.41 HOURS

Fig. 4

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HUMAN METABOLITES OF AMONAFIDE

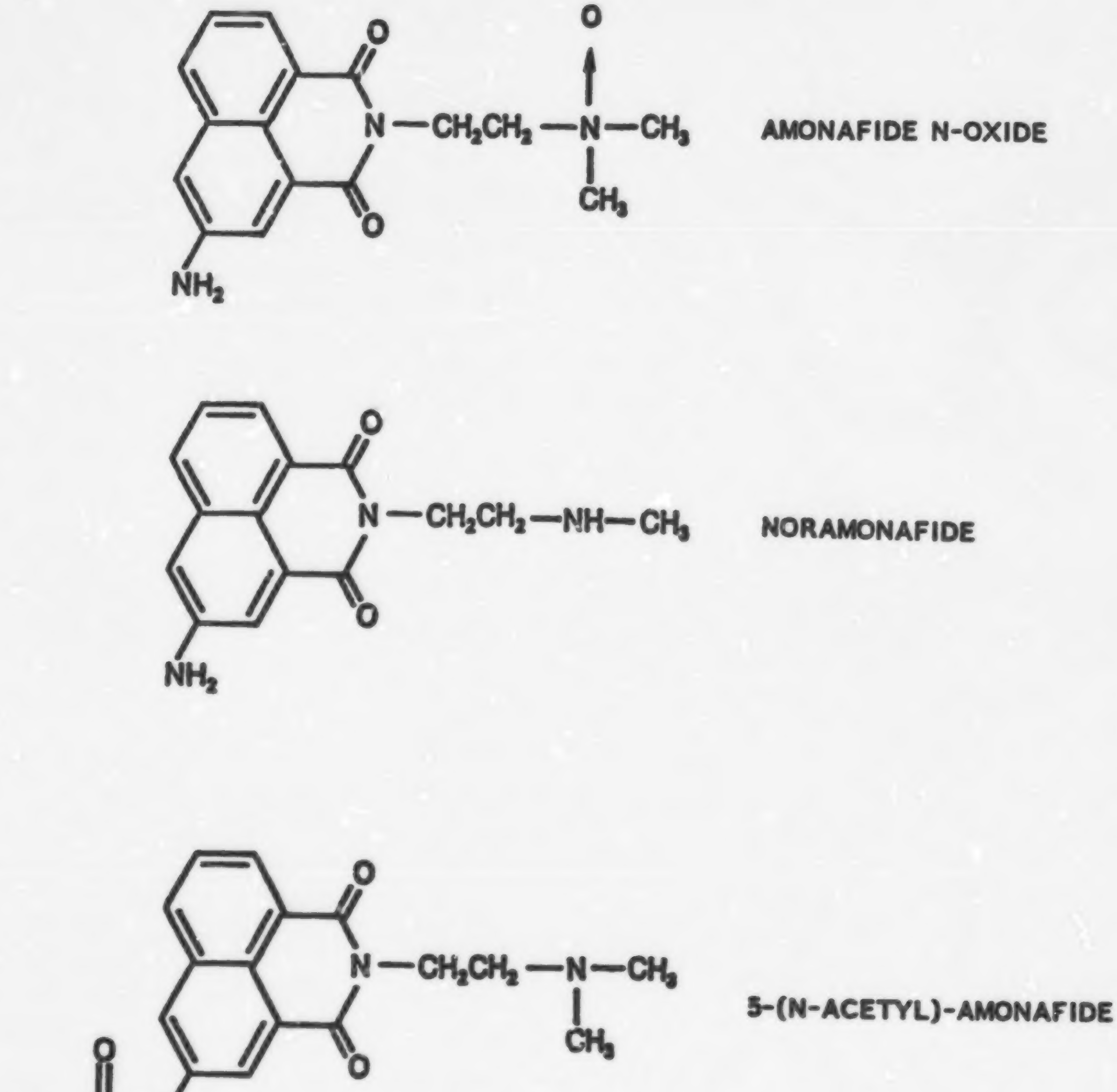
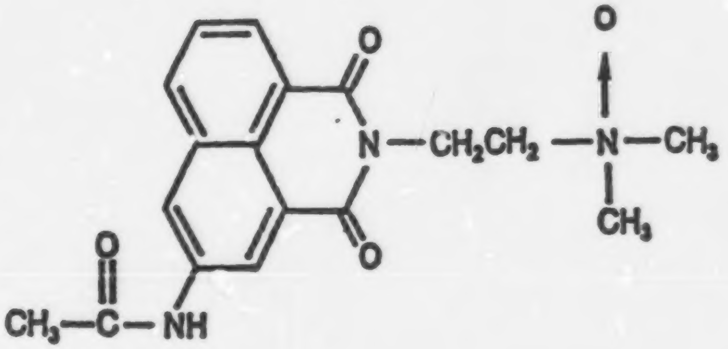
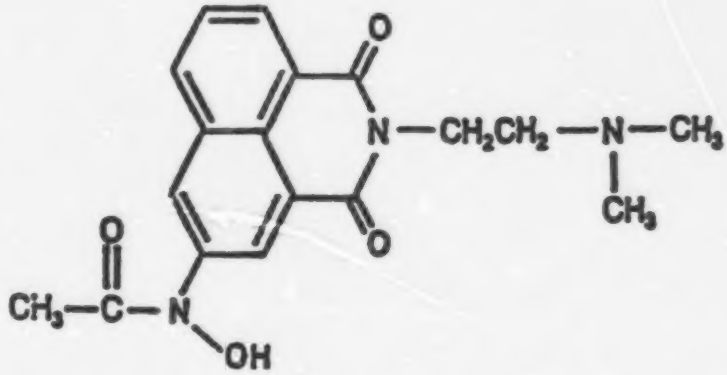

O
O
N—CH$_2$CH$_2$—N—CH$_3$
CH$_3$
O
NH$_2$
AMONAFIDE N-OXIDE
O
O
N—CH$_2$CH$_2$—NH—CH$_3$
NH$_2$
NORAMONAFIDE
O
O
N—CH$_2$CH$_2$—N—CH$_3$
CH$_3$
CH$_3$—C—NH
O
5-(N-ACETYL)-AMONAFIDE

Fig. 5

HUMAN METABOLITES OF AMONAFIDE



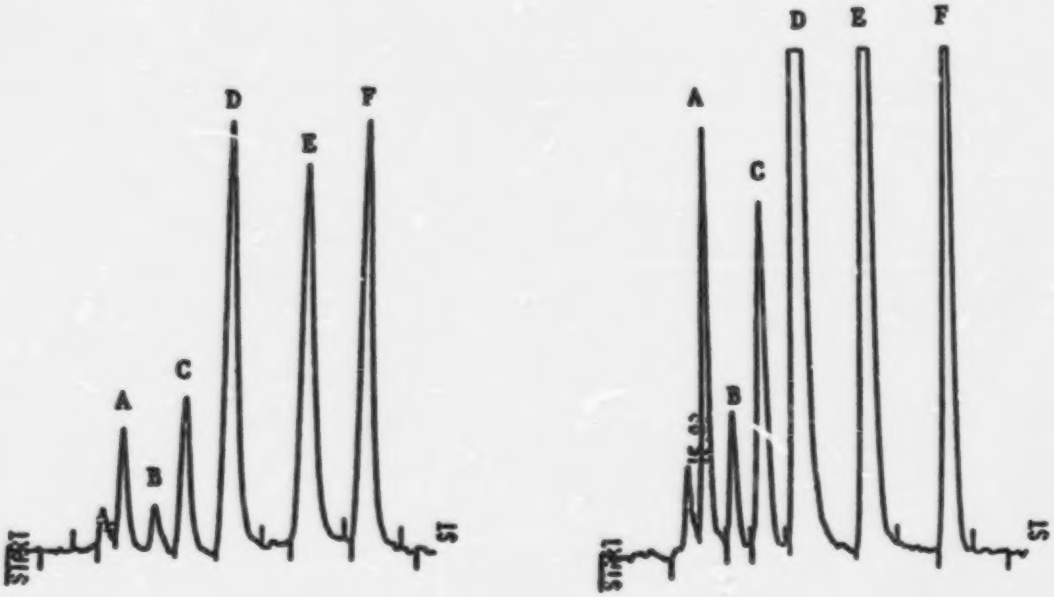
5-(N-ACETYL)-AMONAFIDE N-OXIDE



5-(N-ACETYL, N-HYDROXY)-AMONAFIDE

Fig. 6

CHROMATOGRAMS OF EXTRACTS OF PATIENT PLASMA OBTAINED AT 18 HR FOLLOWING THE IV BOLUS ADMINISTRATION OF AMONAFIDE 165.6 MG/M^2 (LEFT) AND 220.8 MG/M^2 (RIGHT)



CHROMATOGRAPHIC PEAKS:

(A) noramonafide

(B) 5-(N-acetyl)-amonafide N-oxide

(C) 5-(N-acetyl, N-hydroxy)-amonafide

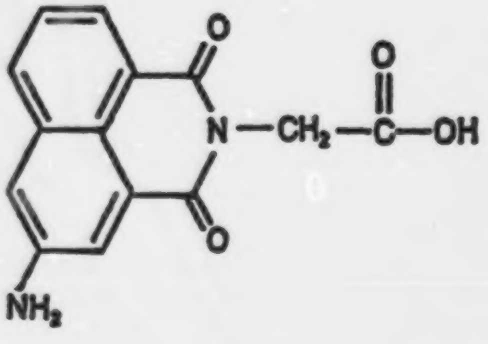
(D) amonafide

(E) 5-(N-acetyl)-amonafide

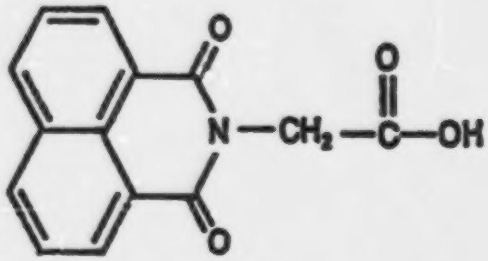
(F) internal standard

Fig. 7

STRUCTURE OF AN ACIDIC METABOLITE OF AMONAFIDE AND OF THE ALDOSE REDUCTASE INHIBITOR ALRESTATIN



5-AMINO-1,3-DIOXO-1H-BENZ[DE]ISOQUINOLINE-2(3H)-ACETIC ACID



ALRESTATIN

Fig. 8

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Phase I Trial of Nafidimide in San Antonio

Slide. Our study utilized a single dose every 28 day schedule of Nafidimide. The mode of administration was infusion in 100 cc's of 0.9 % sodium chloride over 30 minutes. We started at 1/3 of 1/10 of the LD10 and the escalation scheme is shown here from 18 to 660 mg/m^2.

Slide. Fourteen patients receiving 25 courses have been entered on study with a median age of 64 and a median performance status of 1 Two patients had no prior chemotherapy, 12 had prior chemotherapy and radiotherapy.

Slide. Half the patients on this study had non small cell lung cancer with a distribution of other tumor types.

Slide. The dose escalation scheme and the accrual of each dose level are shown here with three patients on each level except the initial dose level which we skipped because our colleagues had patients at higher doses.

Slide. The toxicities noted with Nafidimide included nausea and vomiting in three patients. This nausea and vomiting in the patients reporting this were not severe. A funny taste has been reported by three patients and other toxicities including tinnitus, dizziness and headache. These have been sporadic.

Slide. Of note is that we have seen hints of possible anti tumor effect in two patients. The first patient is a 69 year old male with adenocarcinoma of unknown primary (probably lung). He's had a partial response by CAT scan which is ongoing at three months. The second patient is a 68 year old male with prostate cancer who had a remarkable decrease in pain with no change in scans or enzymes.

Slide. Our plans are to continue dose escalations.

Patient Characteristics

Number of patients	14	(25 courses)
Men/women	12/2	
Age in years		
Median	64	
Range	(54-73)	
Median performance status	1	(0-3)
Number of patients without prior therapy	2	
Number of patients with prior therapy	12	
Chemotherapy only	7	
RT + chemotherapy	5	

San Antonio Phase I Trial of Nafidimide

1) Schedule: Single dose every 28 days
2) Mode of administration:
   Infusion in 100 cc of 0.9% NaCl over 30 minutes
3) Start at 1/3 of 1/10 of LD_{10}
4) Escalation scheme: 18, 220, 293, 390, 519, 660

Tumor Types in Patients

Non small-cell lung	7
Kidney	2
Prostate	2
Melanoma	1
Pancreas	1
Colon	1

The Escalation Schema/Accura

Dose (mg/m^2)	No. of patients
18	1
220	3
293	3
390	3
519	3
660	1

Toxicities Noted with Nafidimide

Toxicity	# of Patient (dose level)	
Nausea and vomiting	3	(220, 293, 293)
Taste "funny"	3	(200, 220, 390)
Tinnitus	2	(390, 519)
Dizziness	1	(519)
Headache	1	(220)
Circumoral numbness	1	(390)

Responses

1) 69 yo male with adenocarcinoma of unknown primary (probably lung). Partial response by CAT scan - ongoing at 3 months.
2) 68 yo male with prostate cancer remarkable decrease in pain with no change in scans or enzymes (Alk phosph, acid phosph).

Plan

1) Continue dose escalations

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Choice of 24° Infusion Schedule

- schedule dependence vs L12 10
- single IV dose produced 37% increase lifespan
- same dose IP in 8 doses/24 hours produced 74% increase lifespan

Choice of Starting Dose

1/10 - single day MELD-10 = 18.4 mg/m^2

- transient renal and hepatic toxicity seen in animals at this dose
- starting dose 1/30 MELD-10 = 6.1 mg/m^2

1. Advanced cancer beyond cure or effective palliation with standard therapy.
2. Adequate hematologic status
WBC $\geq$ 4000, HGB $\geq$ 10, PLTS $\geq$ 130,000
3. Adequate hepatic function
non-elevation of direct bilirubin, alkaline phosphatase, or SGOT < 3x normal
4. Adequate renal function
creatinine $\leq$ 1.5
5. Perforance status 0, 1, 2
6. No CNS metastases
7. No significant heart disease
8. Recovery from prior therapy
9. Informed consent

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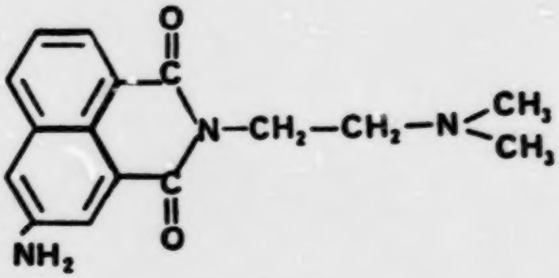

Planned Dose Escalation for New Patient Entries In Absence of Toxicity

Dose Level	Nafidimide (Total dose to be divided into 3 doses in 250 ml NS infused over 8 hours x 3)
1	6.1 mg/m^2
2	18.4 mg/m^2
3	100 mg/m^2
4	200 mg/m^2
5	400 mg/m^2
6	600 mg/m^2
	& 50% thereafter until first evidence of toxicity*

*At first evidence of a toxicity of a type and degree of severity that could be dose limiting, dose will not be escalated more than 25% in new patients.

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	PATIENT						
Level	1	2	3	4	5	6	7
6.1 mg	x	x	x	-	-	-	-
18.4 mg	x	prog	prog	x	x	x	-
36.8 mg	x			x	x	prog	-
73.6 mg	x			x	-		-
100 mg	prog			prog	x		x

No interval or acute toxicity
No objective responses

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PHASE I PHARMACOKINETIC STUDY OF NAFIDIMIDE BY 24 HOUR INFUSION

Mayo Clinic

1. Assay

A sensitive and specific assay for nafidimide has been developed where plasma is injected directly onto the hplc without the need for prior extraction or sample work up (Powis et al., J. Chromatogr. 345:402-407, 1985). The plasma is first passed through a 50 x 4 mm precolumn packed with RP-8 Perisorb® which adsorbs drug and its metabolites. The precolumn is then flushed with water for 3.5 min at 3 ml/min to remove protein to waste. Using an automatic switching valve, drug and metabolites are displaced from the precolumn onto an RP-18 analytical column using a 10 min linear gradient of 0-100% methanol in 10 mM sodium phosphate buffer, pH 8.1, at a flow rate of 1.5 ml/min. Eluting compounds are detected by their fluorescence at an excitation wavelength a 254 nm with an emission filter of 470 nm. Characteristics of the assay are as follows:

Sample volume: 0.5 ml plasma, injected directly

Precolumn: 50 x 4 mm RP-8 Perisorb

Analytical column: 25 cm RP-8, 4 µm LiChrospher II

Solvent system: 0-100% methanol in 10 mM sodium phosphate, pH 8.1 over 10 min

Flow: 1.5 ml/min

Time: total time 22 min (analytical run 10 min)

Limit: lower limit (5 x background) 2.5 ng/ml
upper limit, 10 µg/ml without sample dilution

Coefficient of variation: 1 µg/ml 1.1%
0.1 µg/ml 1.0%
0.01 µg/ml 16.5%

Figure 1 shows a sample of human plasma to which nafidimide has been added at 10 ng/ml. Metabolite peaks could be detected in the plasma of dog receiving nafidimide 4.6 mg/kg (100 mg/m^2) by i.v. bolus injection (Figure 2).

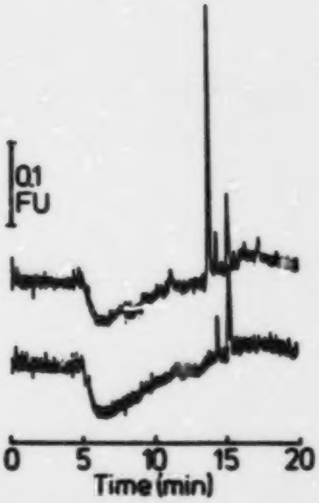

Figure 1. Chromatogram of nafidimide added to human plasma at 10 ng/ml (upper trace) and blank plasma (lower trace).

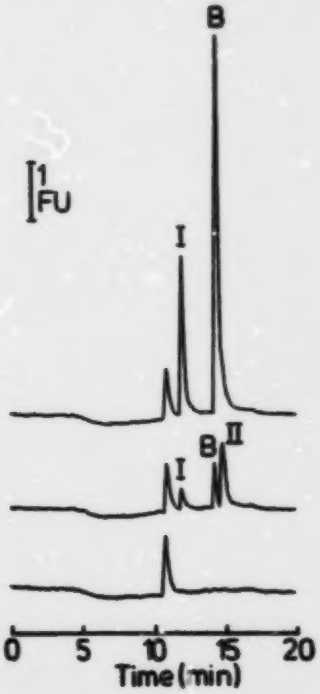

Figure 2. Chromatogram of plasma from a dog given nafidimide 4.6 mg/kg by i.v. bolus. Upper trace, plasma 1 hour after giving drug; middle trace, plasma 4 hour after giving drug; lower trace, plasma before giving drug. Peaks: B = nafidimide, I and II = metabolites.

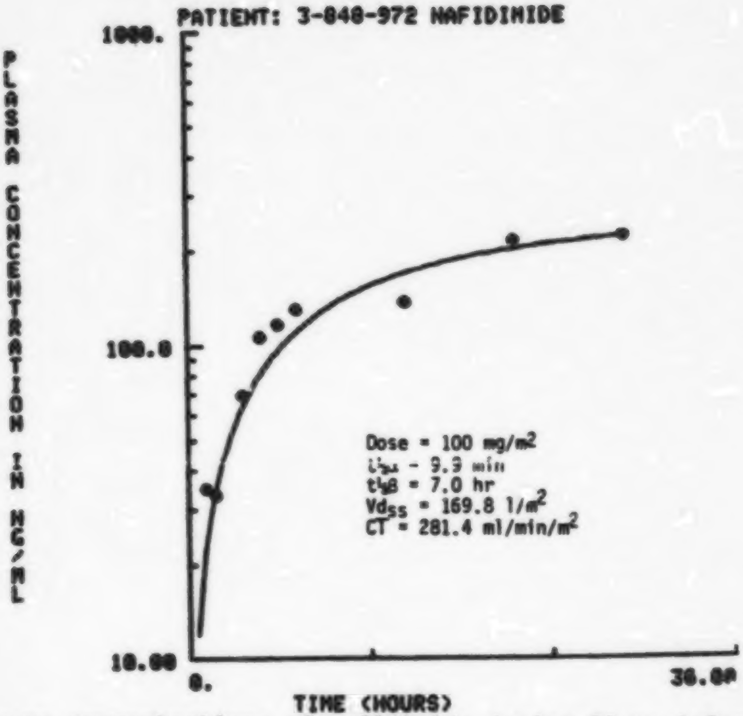

Figure 3. Plasma concentrations of nafidimide during 24 hr infusion at a dose of 100 mg/m^2. Points are the observed values, the continuous line is the computer fit to the data.

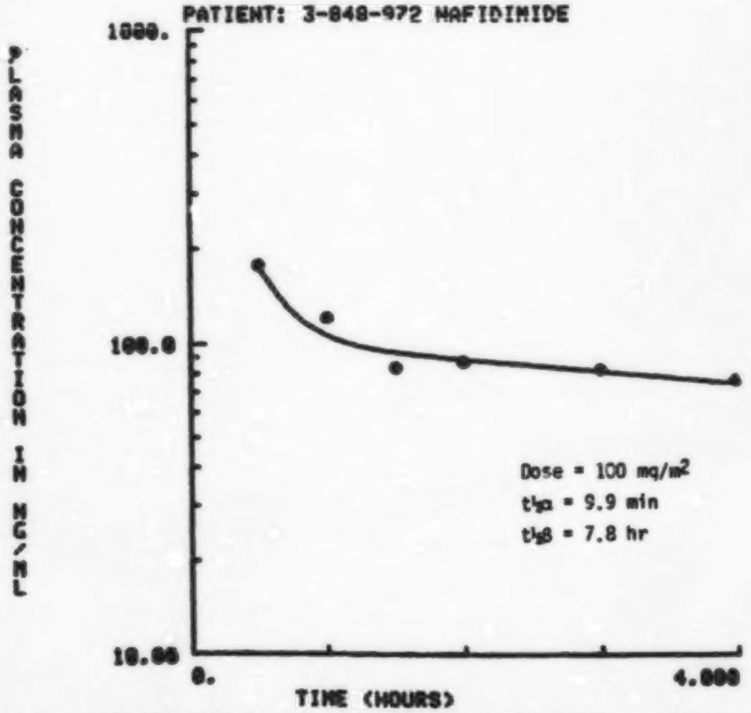

Figure 4. Plasma concentrations of nafidimide following a 24 hr infusion at a dose of 100 mg/m^2 in the same patient as Figure 3. Points are the observed values, the continuous line is the computer fit to the data.

IMPACT OF PHASE I CLINICAL TRIALS ON THE QUALITY OF LIFE AND SURVIVAL OF PATIENTS WITH CANCER

SPECIFIC AIM

To determine the impact of Phase I clinical trials on the quality of life and performance status of patients with cancer.

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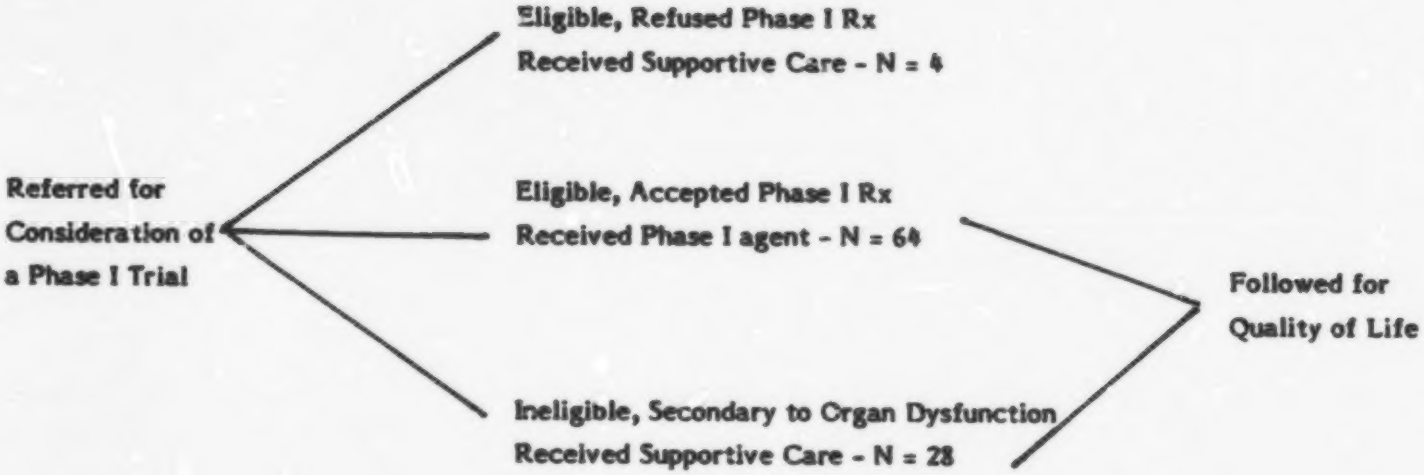
STUDY DESIGN


Referred for
Consideration of
a Phase I Trial
Eligible, Refused Phase I Rx
Received Supportive Care - N = 4
Eligible, Accepted Phase I Rx
Received Phase I agent - N = 64
Ineligible, Secondary to Organ Dysfunction
Received Supportive Care - N = 28
Followed for
Quality of Life

STUDY PARAMETERS

---

1. Performance Status
(SWOG Criteria)

2. Quality of life
(Linear Analogue Self
Assessment Scale - LASA)

Pretreatment
and Q 3-4 weeks

QUALITY OF LIFE
LASA SCALE VARIABLES

PERFORMANCE
- Level of activity
- Ability to perform housework/job
- Social activities

PERSONAL ATTITUDES/BELIEFS
- Well being
- Mood
- Level of anxiety
- Hope
- *Treatment helping

SYMPTOMS
- Pain
- Nausea
- Appetite
- *Chemotherapy side effects

SUPPORT
- Family
- Doctors/Nurses

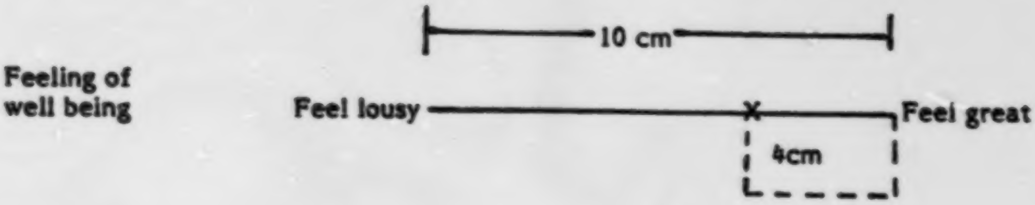
---

* Completed by patients receiving Phase I agents only.

QUALITY OF LIFE
LASA SCALE

1. Feeling of well being — feel lousy ________________________ feel great
2. Level of activity — doing nothing ________________________ very active
3. Pain — much pain ________________________ no pain

QUALITY OF LIFE
LASA SCALE


10 cm
Feeling of well being
Feel lousy
X
Feel great
4cm

Mean LASA = Addition of individual LASA scores divided by the total number responded items.

PATIENT CHARACTERISTICS

Sex

Age

Performance Status

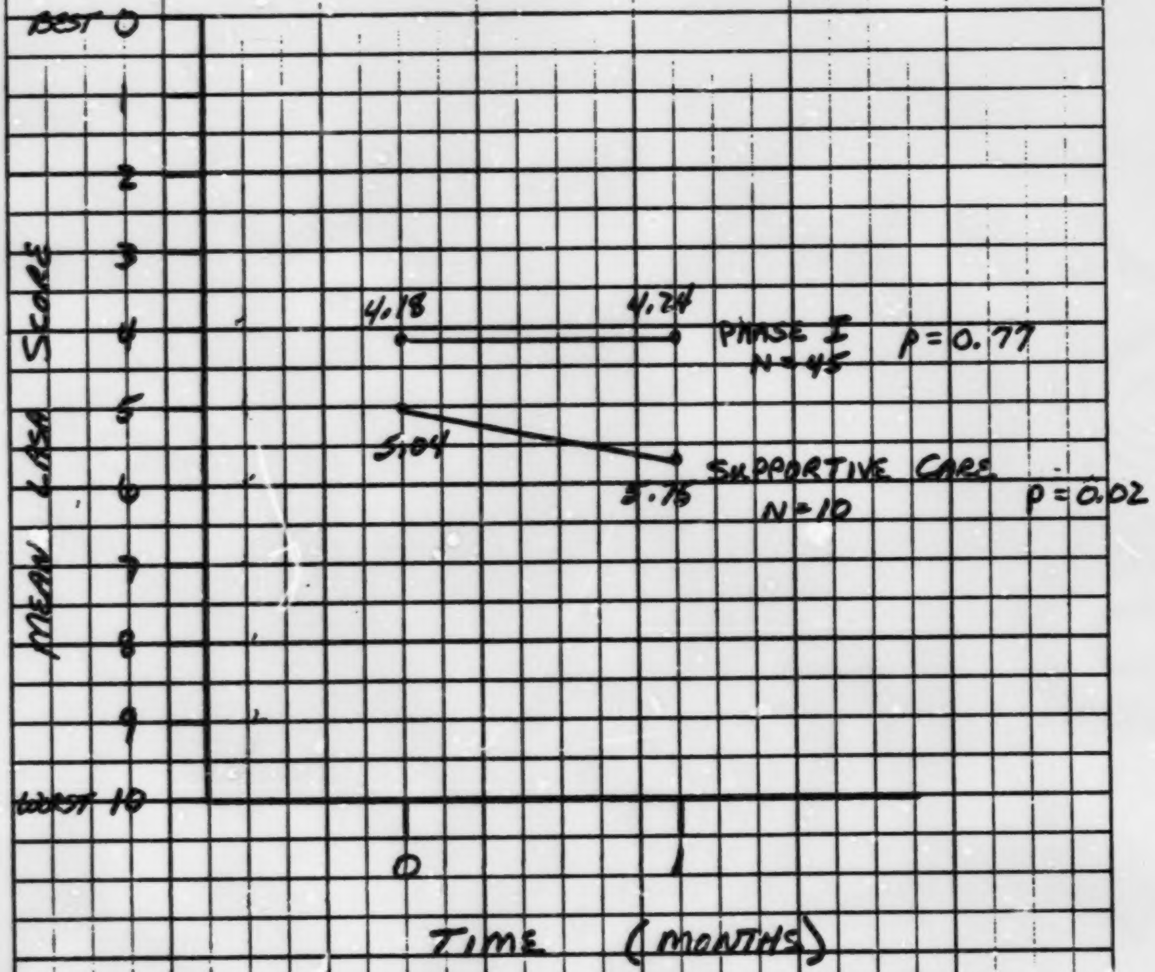
Prior Treatment

Tumor Type

STUDY POPULATION

Parameter	Phase I N = 64	Supportive Care N = 28
# Evaluable	45	10
# Unevaluable	19	18
- Progressive illness/weakness	12	11
- Death	5	6
- Refused participation	2	2

OVERALL QUALITY OF LIFE



INDIVIDUAL QUALITY OF LIFE (LASA) SCORES AT PRESTUDY

Characteristic	Phase I N = 45	Supportive Care N = 10
	Mean LASA Score	
PERFORMANCE		
Level of Activity	6.6	7.0
Ability to Work	6.6	7.8
Social Activity	8.0	7.7
PERSONAL ATTITUDES/BELIEFS		
Hope	1.3	2.9
Treatment Helping	2.3	N/A
Level of Anxiety	3.8	6.5
Mood	4.2	6.1
General Well Being	5.0	6.2
SYMPTOMS		
Chemotherapy Side Effects	2.3	N/A
Nausea	3.1	3.6
Pain	3.8	5.7
Appetite	5.0	3.8
SUPPORT		
Doctors/Nurses	0.8	1.4
Family	2.0	1.6

CHANGES IN QUALITY OF LIFE FOLLOWING TWO CONSECUTIVE EVALUATIONS

Characteristics	Phase I N = 45	Supportive Care N = 10
	p Value	p Value
General Well Being	NS	NS
Mood	NS	NS
Level of Activity	NS	NS
Pain	NS	NS
Nausea	NS	NS
Appetite	NS	0.03
Ability to Work	NS	NS
Social Activity	NS	NS
Level of Anxiety	NS	NS
Family Support	NS	NS
Doctors/Nurses Support	NS	0.03
Hope	NS	NS
Treatment Side Effects	NS	N/A
Treatment Helping ?	NS	N/A

Quality of Life
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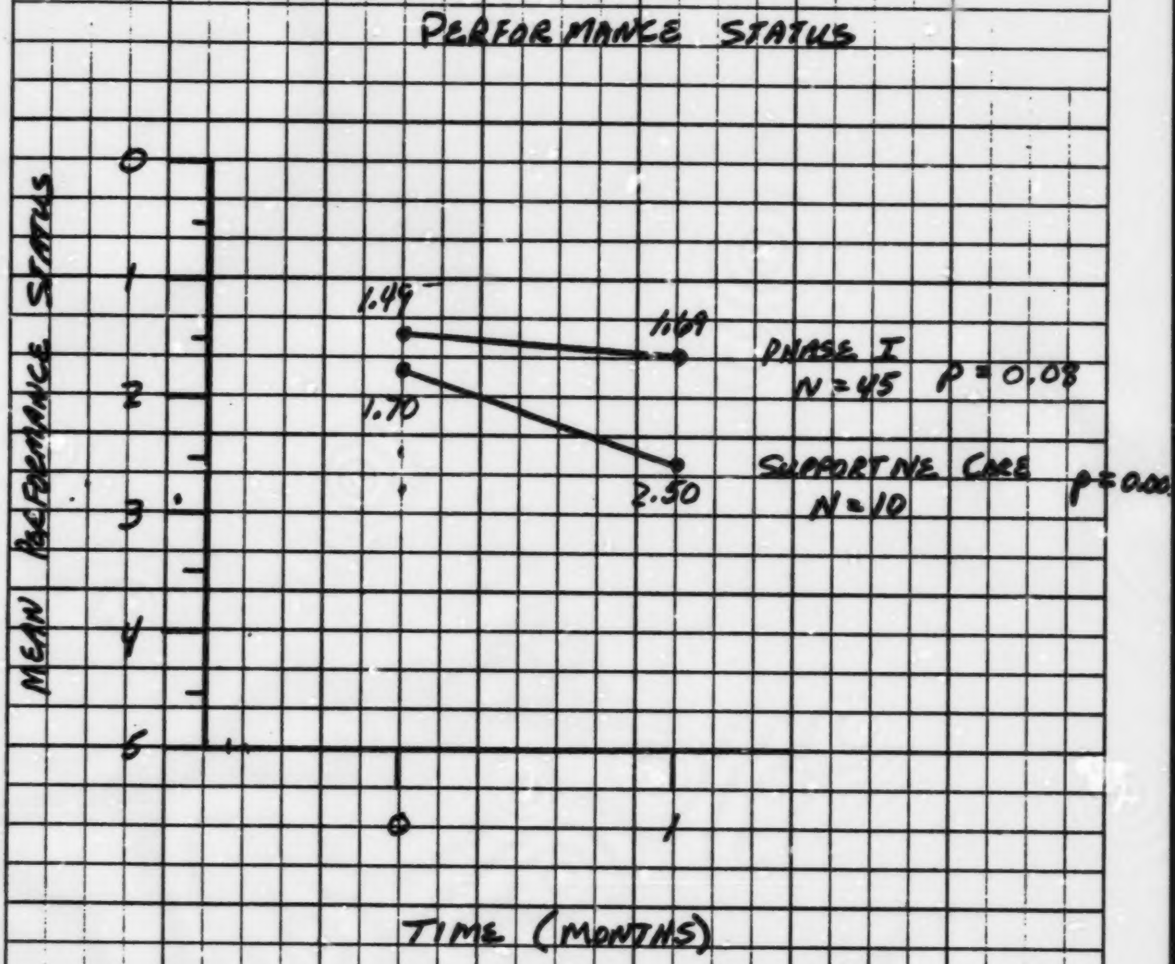

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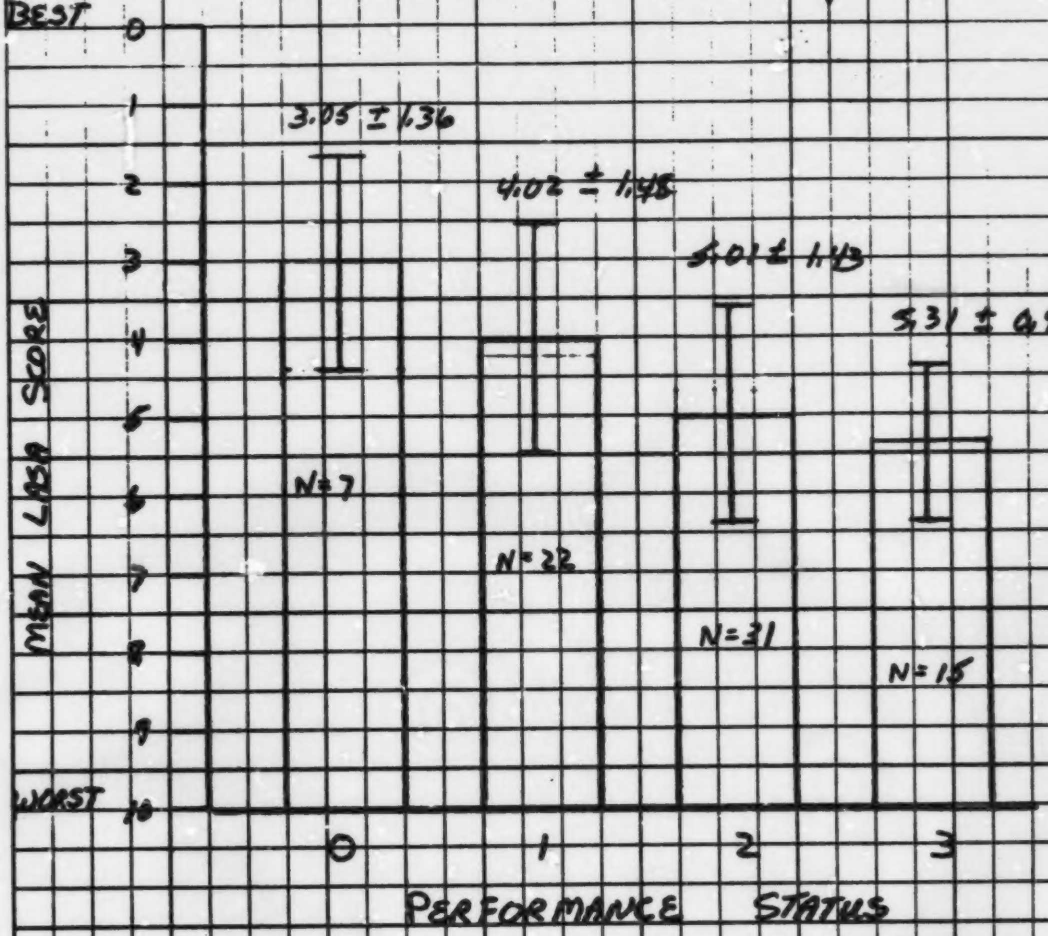
CORRELATION BETWEEN QUALITY OF LIFE & PERFORMANCE STATUS AT PRESTUDY



$r = 0.45$

$p = 0.0002$

SURVIVAL STATUS

Group	Median Months (range)
Phase I	4 (< 1- 23)
Supportive care	1 (< 1- 7)

FACTORS THAT PREDICT SURVIVAL

Variables	p Value
Age	NS
Sex	NS
Treatment	NS
Performance Status	0.0001
LASA Score	0.05

CONCLUSION

Participation in a Phase I clinical trial does not alter one's overall quality of life or performance status following at least one course of therapy.

Tumor Necrosis Factor
Genentech
July 1986

PRELIMINARY RESULTS OF PHASE IA rTNF TRIALS

INSTITUTIONS M. D. ANDERSON HOSPITAL (DR. GUTTERMAN)
MEMORIAL SLOAN-KETTERING CANCER CENTER (DR. OETTGEN)

OVERALL STATUS

• ENTERED		46 (> 200 TREATMENTS)
COMPLETED 4 WEEKS		29
REMOVED EARLY		
TOXICITY		6
FATIGUE	1	
CHILLS, HEADACHE	1	
HYPOTENSION	2	
LOCAL (SQ)	2	
PROGRESSION		2
NON-COMPLIANCE, PROTOCOL VIOLATION		3
OTHER		6
HEPATIC DYSFUNCTION FROM EXTENSIVE TUMOR	1	
SEIZURES WITH CNS METS	1	
SIADH	1	
CORD COMPRESSION	1	
BRONCHIAL OBSTRUCTION FROM MEDIASTINAL TUMOR	1	
LOSS OF VENOUS ACCESS	1	

• HIGHEST DOSES ACHIEVED 250 MCG/M^2 SQ
200 MCG/M^2 IM
200 MCG/M^2 IV

Tumor Necrosis Factor
Genentech
July 1986

STUDY PLAN

SCHEDULE............................TWICE WEEKLY INJECTIONS FOR 4 WEEKS (72-96 HOUR WASHOUT)

DOSE LEVELS.........................MODIFIED FIBONACCI ESCALATION

LEVEL	MICROGRAMS/M^2
1	5 (~0.1 MICROGRAMS/KG)
2	10
3	15
4	25
5	35
6	50
7	65
8	80
9	100...MTD AT 25 MCG/M^2 INCREMENTS

EACH PATIENT RECEIVES TREATMENT OVER FOUR SEQUENTIAL DOSE LEVELS. FIRST TWO PATIENTS BEGIN AT LEVEL 1, NEXT TWO AT LEVEL 2, ETC.

ROUTE OF ADMINISTRATION..............ALTERNATING INTRAVENOUS BOLUS AND SUBCUTANEOUS FOR EACH DOSE LEVEL.

Phase I Trial

rTNF-Alpha
3 X/Week
Intravenous Infusion, 30 minutes

University of Wisconsin Clinical Cancer Center

J Grem
R Brown
E Borden
J Willson
D Trump

Phase I Trial
rTNF, 3 X/Week
Dose Escalation Plan

Level	Dose
1	5 mcg/sq m
2	10 mcg/sq m
3	25 mcg/sq m
4	50 mcg/sq m
5	75 mcg/sq m
6	100 mcg/sq m
7	125 mcg/sq m
8	150 mcg/sq m

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Tumor Necrosis Factor
T86-0001
Wisconsin
July 1986

PATIENT CHARACTERISTICS
n = 12

1) Sex	9 male/3 female	
2) Performance status	ECOG 0--7 I--4 II--1	
3) Age--median (range)	55 (28-77)	
4) Prior therapy	None	4
	Prior chemo	3
	Prior XRT	1
	Both	4
5) Tumor type	Colorectal	6
	Non-small cell lung	1
	Bladder	1
	Stomach	1
	Nasopharyngeal	1
	Breast	1
	Soft tissue sarcoma	1

Dose	Number of patients	Highest temperature median (range)	Rigors			Fatigue, malaise	Weight loss
			Mild	Moderate	Severe		
5 mcg/m^2	3	39.5 (39-40.2°)	0	0	3	2	0
10 mcg/m^2	5	38.1 (37.3-39.2°)	1	0	3	0	0
25 mcg/m^2	4	37.8 (37.5-38.8°)	3	1	0	0	0

HEMATOLOGIC TOXICITY

Dose	Number of patients	Number of evaluable ~~patients~~ courses	Nadir WBC ($x10^3$) median (range)	Nadir poly ($x10^3$) median (range)	Nadir Plt ($x10^3$) median (range)	Nadir Hgb (gm/dl) median (range)
5 mcg/m^2	3	4	6.75 (5.9-9.2)	4.05 (3.7-5.6)	327 (256-348)	10.4 (9.4-11.1)
10 mcg/m^2	5	7	5.30 (4.7-7.2)	4.10 (2.24-6.1)	318 (205-419)	10.4 (9.6-12.4)
25 mcg/m^2	4	6	6.30 (4.4-8.0)	4.50 (2.4-5.6)	307 (251-445)	12.0 (10.6-13.4)

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Allergic 1 patient at 25 mcg/m^2 experienced an episode of acute respiratory distress associated with cyanosis of the nailbeds. The patient remained conscious, and the BP and pulse were normal. The patient improved within 3 minutes after administration of supplemental oxygen. EKG rhythm strip: NSR CXR: no change Lungs: clear No JVD COR: Normal heart sounds.

	Arterial blood gases	pO_2 (mmHg)	pCO_2 (mmHg)	pH
	100% O_2	224	31	7.34
30 min.	50% O_2	187	29	7.38
120 min.	3 L/min	80	30	7.36
300 min.	Room air	78	31	7.41

ENT exam the next morning: normal
Skin tests performed two weeks later (Scratch and intradermal) to dilute and undilute rTNF: No reaction.

Other Potential Toxicities

1. **Pulmonary** 1 pt (10 mcg/sq m) with pulmonary metastases, dyspnea with rigors
1 pt (25 mcg/sq m) with pleural effusion pretherapy developed increasing localized pulmonary infiltrate/atelectasis, dyspnea and deteriorating PFT's

	Pre	2 mo
FVC	4.46 L	2.56 L
FEV	3.66	2.16 L
DL_{CO}	28.7 (79% predicted)	16.15 (45%)
DL/VA	6.84 (124% predicted)	6.2 (123%)

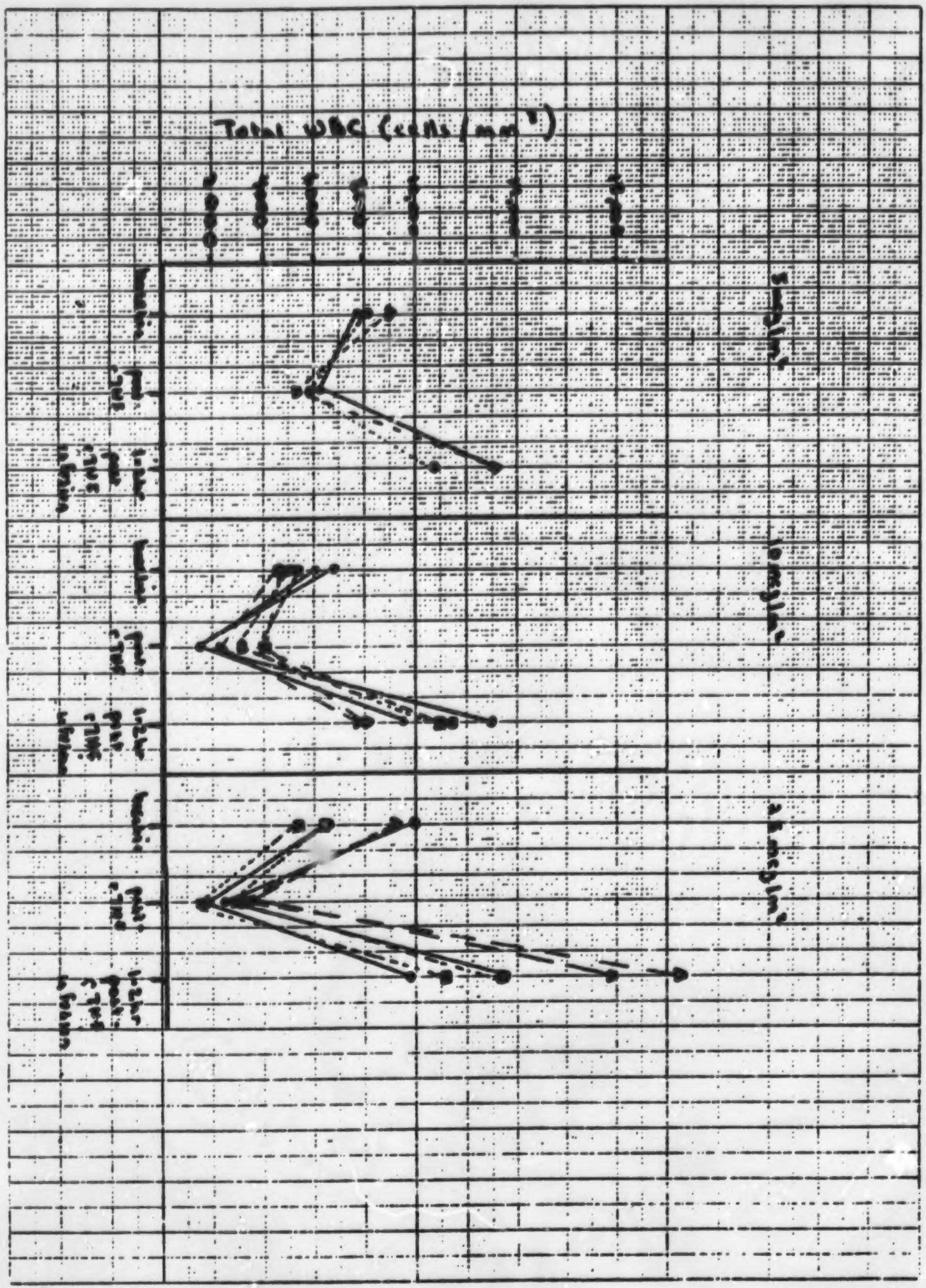
Bronchoscopy/biopsy - no tumor/no pneumonitis

2. **Hypotension** 1 patient, 25 mcg/sq m, minimally symptomatic (132/80 → 98/60 following 6th injection only)

3. **CNS** 1 patient, confusion following 11th injection at 10 mcg/sq m; in setting of substantial disease progression and increasing narcotics - persisted despite cessation TNF

4. **GU** 1 patient, transient elevation of creatinine to 2.1 mg/dL on day 14 of therapy. Returned to normal despite continued TNF.

1 patient with rapidly progressive bladder cancer experienced a rise in creatinine to 3.5 mg/dL five days following 11th TNF injection (10 mcg/sq m). Progressive disease, contrast scans (X2) and disease-related hydronephrosis (existent pre Rx) contributed.



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Tumor Necrosis Factor
T86-0001
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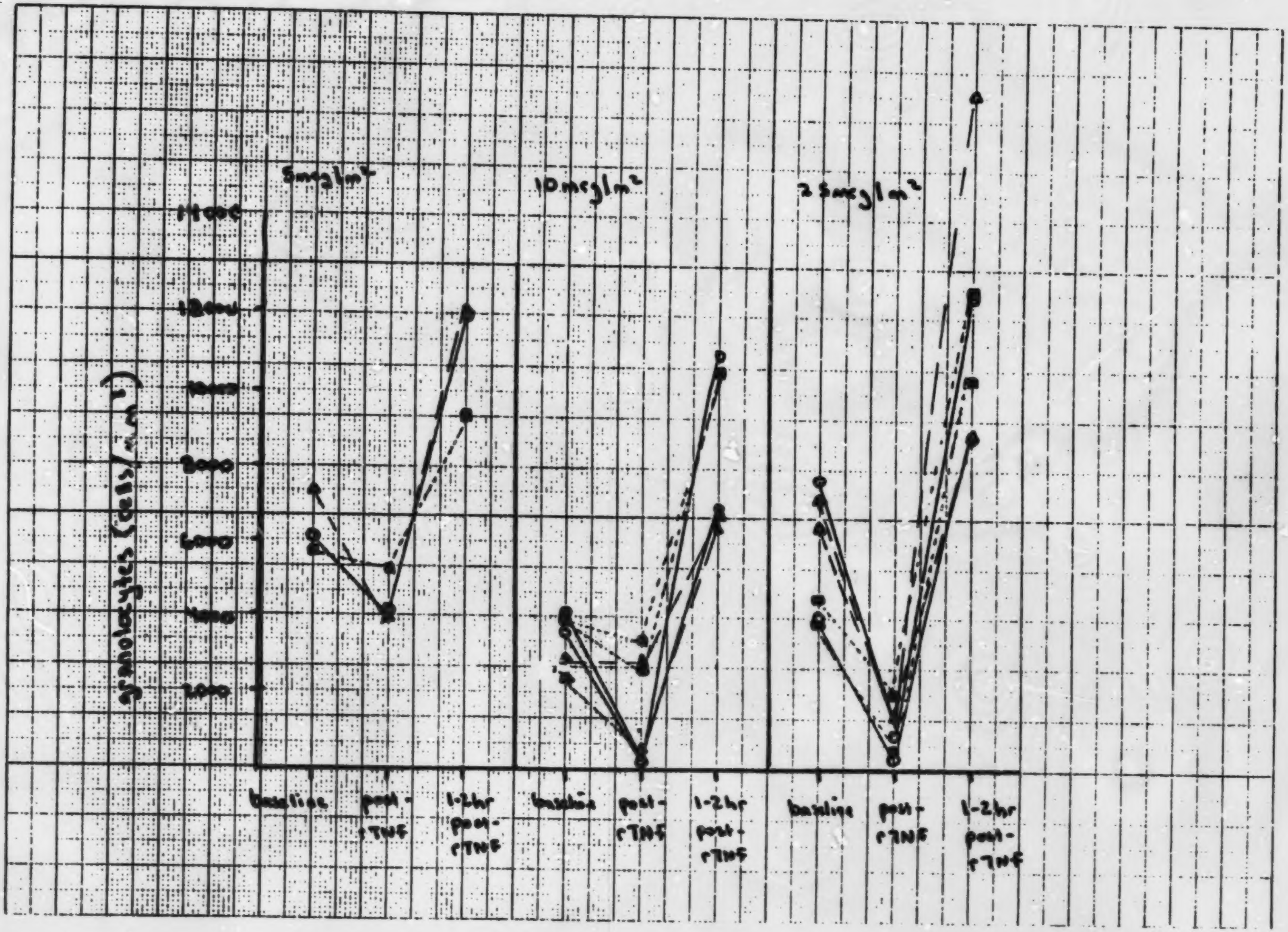

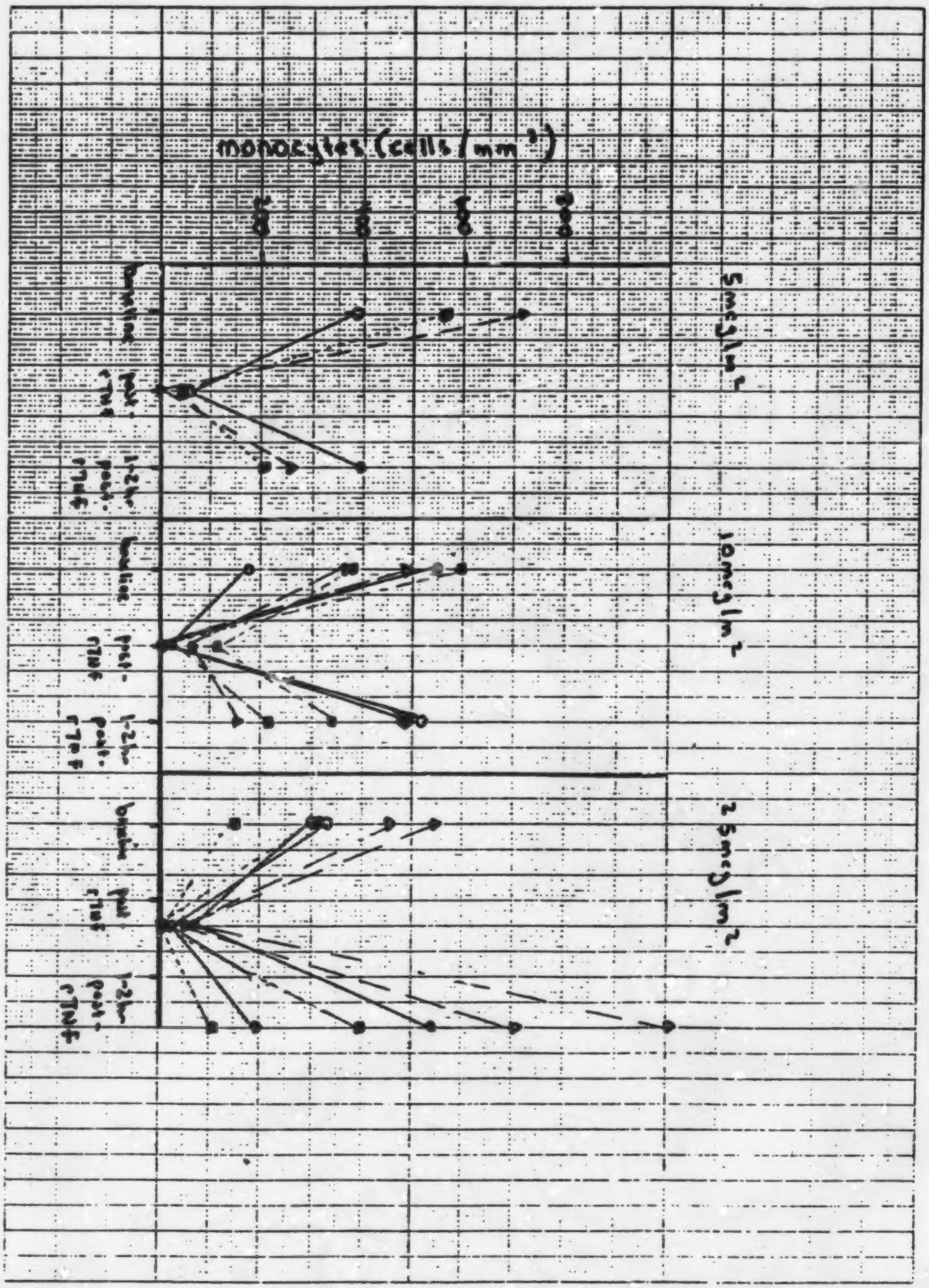
monocytes (cells/mm³)
200
400
600
800
5mcg/m²
10mcg/m²
25mcg/m²
baseline
post-rTNF
1-2hr post-rTNF
baseline
post-rTNF
1-2hr post-rTNF
baseline
post-rTNF
1-2hr post-rTNF

Tumor Necrosis Factor
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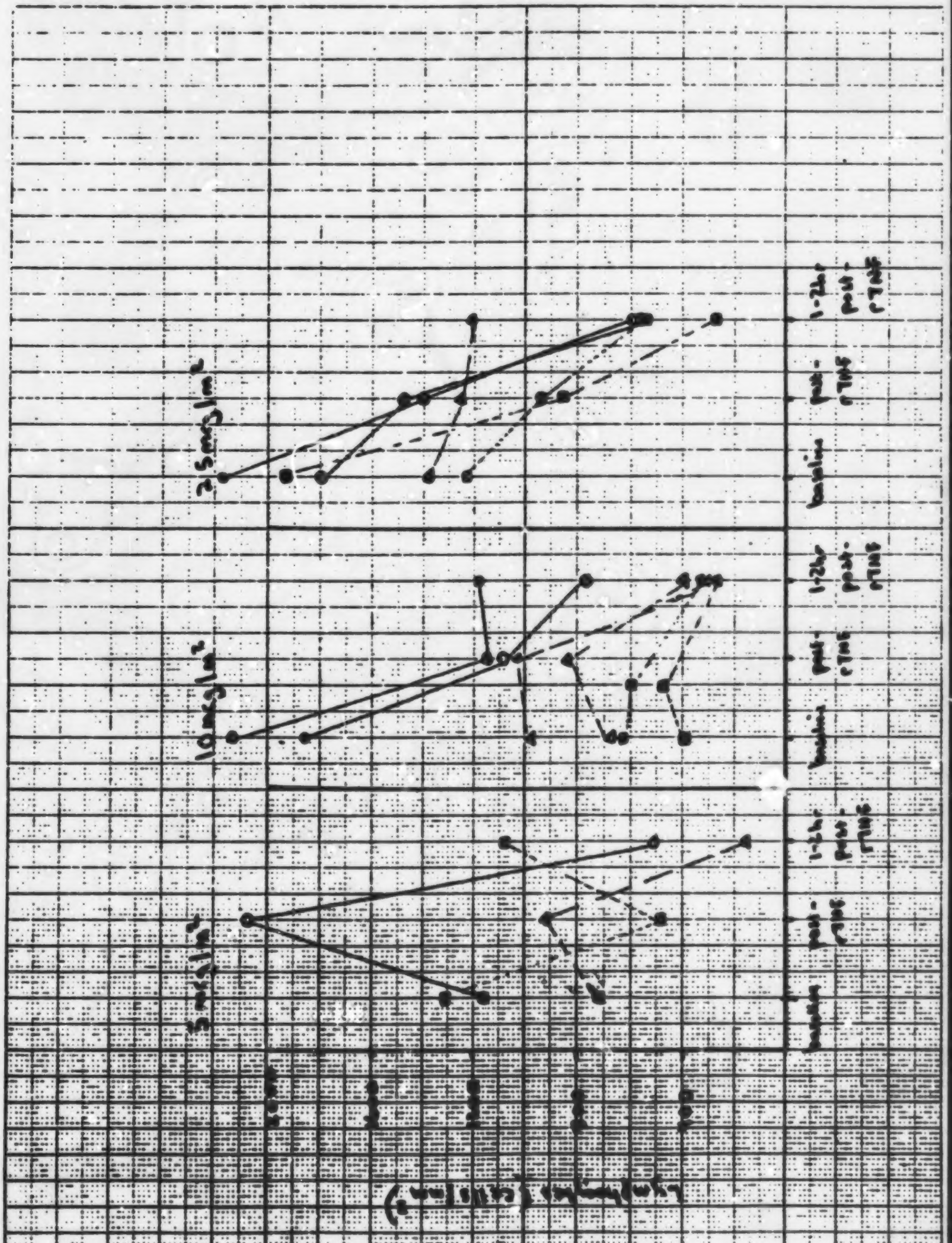

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EFFECT OF rTNF ON PLASMA LIPIDS

Rationale:

rTNF has been shown to induce key enzymes involved in lipoprotein degraditive metabolism. In addition, Patients receiving β_{ser} IFN at the UWCCC were shown to have a significant increase in plasma triglycerides and significant decreases in plasma cholesterol over the first 10 days of treatment.

Results:

Samples are obtained at baseline, on days 2, 4, and 7, and weekly thereafter until 4 weeks post-therapy. Preliminary results in the first 12 patients indicate that no major changes in plasma cholesterol or triglyceride levels have been observed.

EFFECT OF rTNF on TRYPTOPHAN METABOLISM

Rationale:

Bacterial lipopolysaccharide has been shown to induce tryptophan degradation when injected intraperitoneally in mice. This effect occurs through induction of indoleamine 2,3 dioxygenase (IDO), the rate-limiting enzyme in tryptophan catabolism. LPS is known to increase interferon titres, and it is thought that interferon directly induces IDO, thereby enhancing tryptophan degradation.

Clinical studies at the UWCCC have shown that plasma tryptophan levels decrease and urinary tryptophan metabolites increase in patients receiving beta/gamma interferon and in patients receiving interleukin-2.

Results:

Serum and 24-hour urine samples are being collected daily for the first week of therapy, and then weekly thereafter. The urinary kynurenin is measured by an automated steam volatile method.

In the first 11 patients entered on study, no changes in the 24-hour urinary kynurenin has been seen.

CONCLUSION

1. Rigors and fever are seen universally following rTNF administration.

2. No dose-limiting toxicity has been seen. Patient entry continues with plans to monitor lipid and tryptophan metabolism as well as "BRM parameters" at the MTD.

Tumor Necrosis Factor
T85-0252
Mayo Clinic
July 1986

PHASE I TRIAL

RECOMBINANT HUMAN TUMOR NECROSIS FACTOR (rTNF)

DAILY x 5 DAYS Q. 4 WEEKS

rTNF IN 50 ML ISO NACL WITH

2 MG/ML HUMAN ALBUMIN OVER 30 MIN

PHASE I rTNF

EXCLUSIONS

HEMATOLOGIC	KNOWN BLEEDING DISORDER WBC < 4000 PLAT < 130,000 HGB < 10
HEPATIC	↑ DIRECT BILI
RENAL	> 1.5 CREATININE
PS	≥ 2 (ECOG)
CARDIAC	NY HEART III OR IV OCCLUSIVE VASCULAR DISEASE

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PHASE I RTNF

PLAN OF ESCALATION

LEVEL	DOSE (UG/M^2/DAILY X 5 DAYS)
1	5
2	10
3	25
4	50
5	75
6	100
7	125

PHASE I RTNF

DAILY DOSE (UG/M^2)	NO. PTS./COURSE 1ST	 2ND	 3RD	 4TH
5	3	3	1	1
10	4	1	1	

PHASE I rTNF

DAILY DOSE (µG/M²)	NO. COURSES INITIAL	TOTAL	TOXICITY
5	3	8	RIGOR; FEVER (ALL)
10	3	6	RIGOR; FEVER (ALL) HYPOTENSION (1) LEUKOPENIA (1) THROMBOCYTOPENIA (1)

PHASE I rTNF

TOXICITY

HYPOTENSION (10 µG/M^2)

54 Y.O. WOMAN; P.S. 1
3½ HOUR AFTER DAY 5 RX BP 52/30
CORRECTED WITH FLUIDS, NO SEQUELLAE

LEUKOPENIA (10 µG/M^2)
57 Y.O. MAN; P.S. 1; NO PRIOR RX
COURSE: 1 (1300)
2 (3200)
3 (3200)

THROMBOCYTOPENIA (10 µG/M^2)
67 Y.O. MAN; P.S. 1; PRIOR CAP, MTX; RDRX
COURSE: 1 (73,000)

AECC TAXOL PHASE I CI STUDY

WIERNIK, STRAUMAN, EINZIG, DUTCHER, FUKS, WADLER, & SCHWARTZ.

TREATMENT

TAXOL: 24 HOUR CONTINUOUS INFUSION EVERY 3 WEEKS

PRE-MEDICATION

DEXAMETHASONE	20mg.	P.O.	7 AND 14 HOURS PRE-TAXOL
DIPHENHYDRAMINE	25mg	IV	1 HR. PRE-TAXOL
CIMETIDINE	300mg	IV	1 HR. PRE-TAXOL

Taxol
T84-0195
Albert Einstein
July 1986

TAXOL: PHASE I: 24 HOUR IV INFUSION

24 PATIENTS

SEX:	FEMALE	15	
	MALE	9	
MEDIAN AGE (range):		55 (21-83)	
MEDIAN PERFORMANCE STATUS (range):		80 (50-90)	
PRIMARY TUMOR:		MELANOMA	11
		RENAL CELL	5
		ADENOCARCINOMA OF COLON	3
		ADENOCARCINOMA OF LUNG	1
		OVARIAN	1
		SQUAMOUS CELL OF NASOPHARYNX	1
		NEUROFIBROSARCOMA	1
		ADENOCARCINOMA OF UNKNOWN PRIMARY	1
PRIOR TREATMENT*		NONE	2
		SURGERY ONLY	7
		RADIOTHERAPY	6
		CHEMOTHERAPY	12
		1-2 REGIMENS	10
		> 2 REGIMENS	2
		HORMONAL THERAPY	2
		IMMUNOTHERAPY	2

* SOME PATIENTS ARE INCLUDED IN MORE THAN ONE CATEGORY

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DOSE MG/M^2	NO. OF PATIENTS	NO. OF COURSES
125 [2]	1	1
150	5	9
190 [1]	1	1
200	6	22
250	8	14
275	5	10

[1] PATIENT INITIALLY ENTERED AND TREATED AT 250 mg/M^2, DOSE REDUCED BY 25% BECAUSE OF PERIPHERAL NEUROPATHY.

[2] PATIENT INITIALLY ENTERED AND TREATED AT 250 mg/M^2, THEN AT 190 mg/M^2, DOSE REDUCED BY 33% BECAUSE OF PERIPHERAL NEUROPATHY.

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TOXICITIES
(22 EVALUABLE PATIENTS)

DOSE MG/M^2

	150	200	250	275
ANAPHYLAXIS	0/5	0/6	0/6	0/5
RASH/PRURITIS/FLUSHING	0/5	2/6	2/6	3/5
MARROW SUPPRESSION NEUTROPENIA	5/5	6/6	6/6	5/5
	1 Grade 2 1 Grade 3 3 Grade 4	6 Grade 4	1 Grade 3 5 Grade 4	1 Grade 3 4 Grade 4
WBC NADIR				
MEDIAN (Range)	1.5 (0.8-3.4)	1.6 (0.8-4.7)	2.3 (0.7-3.2)	2.0 (1.1-2.3)
PERIPHERAL NEUROPATHY	0/5	3/6	4/6	4/5
		3 Grade 1	1 Grade 1 2 Grade 2 1 Grade 3	1 Grade 1 3 Grade 2

CLINICAL RESPONSES TO TAXOL

TUMOR	NO. OF PATIENTS	DOSE (MG/M^2)	RESPONSE
MELANOMA	2	200	1 PR 1 MR
	6	250	1 PR 1 MR 3 TOO EARLY TO EVALUATE 1 EARLY DEATH
	3	275	1 MR 2 PROGRESSION
RENAL	3	200	2 STABLE 1 PROGRESSION
	2	250	1 STABLE 1 PROGRESSION
COLON	2	150	2 PROGRESSION
	1	275	1 PROGRESSION
LUNG (ADENOCARCINOMA)	1	150	PROGRESSION
OVARIAN	1	150	PROGRESSION
NASOPHARYNGEAL	1	150	PROGRESSION
NEUROFIBROSARCOMA	1	200	PROGRESSION
ADENOCARCINOMA OF UNKNOWN PRIMARY	1	250	PROGRESSION

Taxol
T64-0195
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July 1986

PATIENTS WITH MELANOMA
TREATED WITH TAXOL

DOSE	NO. OF PATIENTS ENTERED	NO OF COURSES
200	2	10 (5,5)
250	6	9 (4,1,1,1,1,1)
275	3	7 (3,2,2)

PATIENTS WITH MELANOMA

CLINICAL RESPONSES

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11 PATIENTS ENTERED

8 PATIENTS EVALUABLE

3 PATIENTS TOO EARLY TO EVALUATE

DOSE (MG/M^2)	PRIOR THERAPY	MEASURABLE DISEASE	RESPONSE	DURATION
200	DTIC/RT	LYMPH NODES	MR	12 WEEKS
200	VELBAN, CYTOXAN, CCNU/RT	NECK MASS, CHEST MASS, LIVER	PR	12 + WEEKS
250[1]	PARTIAL VULVECTOMY	VULVAR LESION, LYMPH NODES	PR	4 + WEEKS
250	BCG	SATELLITE SKIN LESIONS	MR	10 + WEEKS
250	SURGERY	SKIN LESIONS	EARLY DEATH	
250	SURGERY/DTIC	RETROPERITONEAL MASS	TOO EARLY TO EVALUATE	
250	NONE	NECK MASS, LN'S	TOO EARLY TO EVALUATE	
250	NONE	LIVER	TOO EARLY TO EVALUATE	
275	SURGERY	SKIN	MR	4 WEEKS
275	SURGERY	LUNG	PROGRESSION	
275	BCG, DIBROMODUCITAL, BMT, BCNU	SKIN NODULES, ADENOPATHY	PROGRESSION	

[1] TREATED WITH ONE COURSE AT 250mg/M^2, DOSE SUBSEQUENTLY REDUCED TO 190/mg/M^2, THEN 125mg/M^2 BECAUSE OF PERIPHERAL NEUROPATHY, MAINTAINING A RESPONSE.

- Mean residence time (MRT) is analagous to $t\frac{1}{2}$ and is equal to the time required for elimination of 63% of administered dose.
- MRT_{inf} increases with increasing infusion duration.
- $MRT_{iv.}$ is an estimate of the MRT if the drug had been given as an iv. bolus, using the formula:

$$MRT_{IV} = MRT_{INF} - \frac{\text{duration of infusion}}{2}$$

- AUC was calculated with extrapolation to infinity
- β elimination phase was not calculated for 24 hr. infusion study because of inability to measure low concentrations of taxol at later time points.
- Peak plasma conc. was 8-10 fold higher with shorter infusion and AUC was 2.5-4 fold higher with 6 hr. infusion. However elimination parameters ($t\frac{1}{2}$, MRT, renal clearance) were comparable with the two schedules.

ANALYTICAL METHODS

Plasma was obtained from heparinized blood samples by centrifugation. Samples were mixed with acetonitrile, briefly centrifuged, and the supernatant was lyophilized. Samples were analyzed by HPLC using a Whatman Partisil 10 ODS-3 column (4.6 x 250 mm) on a HP1090 Liquid Chromatograph with a diode array detector. Detection was at 227 nm. The mobile phase was a 50 to 85% 10 min. gradient of $\mathrm{CH_3CN}$ to $\mathrm{H_2O/H_3PO_4}$, at a flow rate of 1.5 ml/min. The retention time of taxol was 5.2 minutes.

Taxol concentrations were calculated using a HP3390A integrator by comparison to a standard curve and assuming a taxol extraction efficiency of 75%, which was determined by the use of spiked samples.

Taxol
T84-0195
Albert Einstein
July 1986

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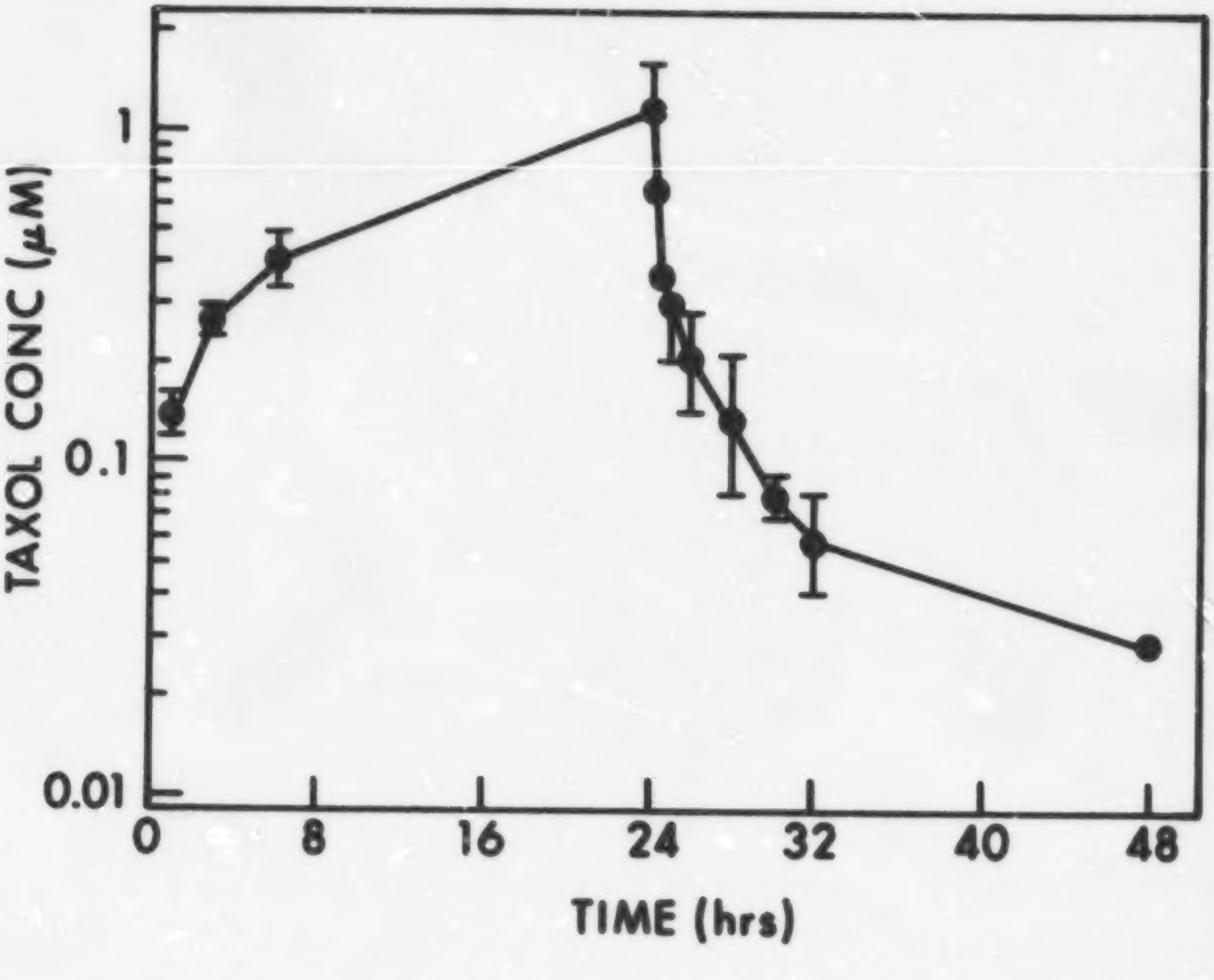

24 hr. infusion
250 mg/m²
Mean ± SEM from 3 patients

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PHARMACOKINETIC PARAMETERS: TAXOL 24 HOUR INFUSION

	DOSE (mg/m^2)		
	200	250	275
Number of patients	3	3	3
Peak plasma concentration (uM)	0.51	0.87	0.94
Area under curve (hr mg/l)	10.6	14.5	13.5
Plasma disappearance, $t_{1/2}\ \alpha$ (hr)	0.59	0.56	0.70
Mean residence time (hr)	15	16	19
Apparent volume distribution$_{SS}$ (l)	125	114	269
Total clearance (ml/min)	592	464	637
Renal clearance (ml/min)	23	6.5	8.9
Urine excretion (% of dose)	3.9%	1.4%	1.4%

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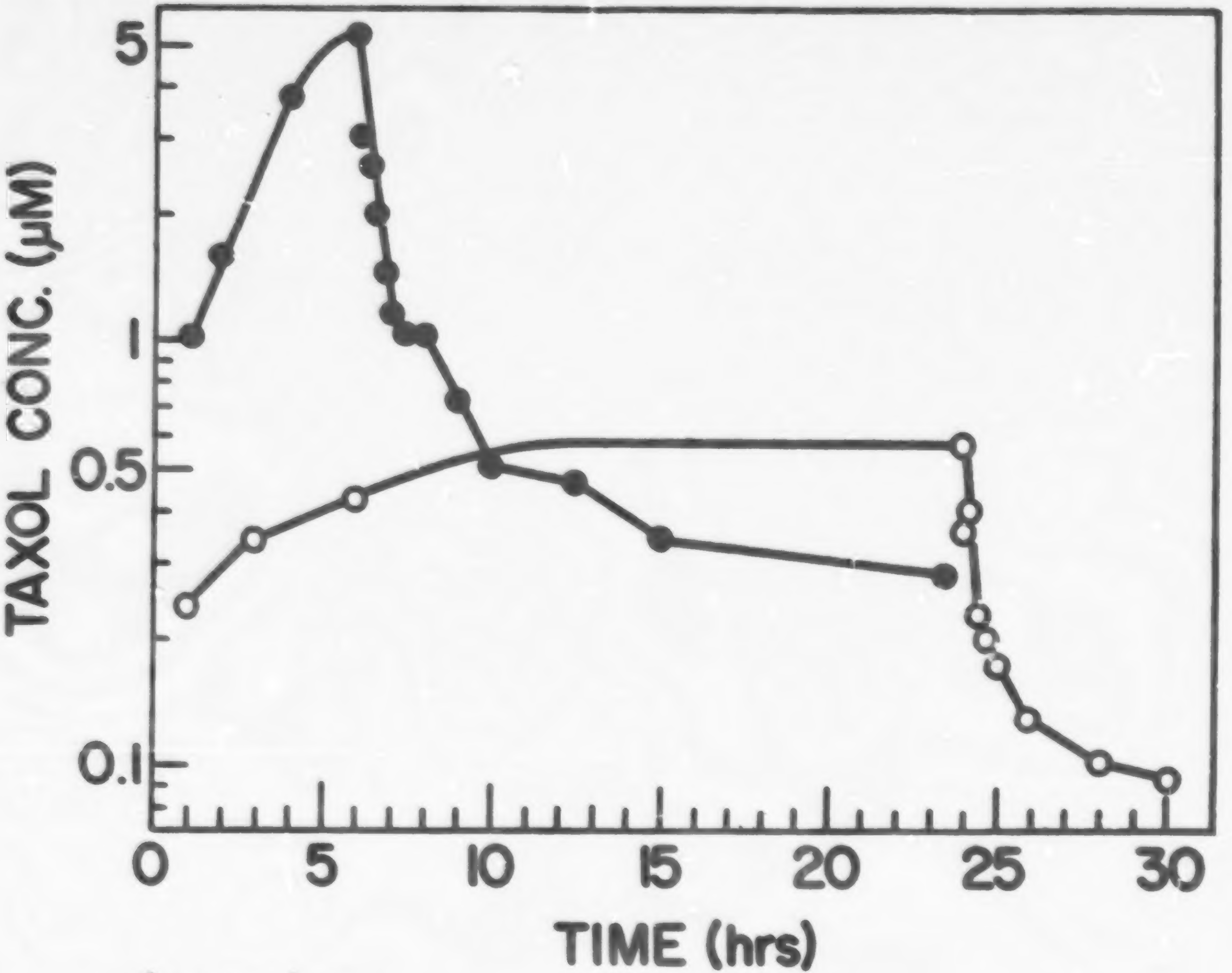

200 mg/m2
● 6 hr. infusion
○ 24 hr. infusion

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PHARMACOKINETIC PARAMETERS: COMPARISON OF 6 HOUR AND 24 HOUR TAXOL INFUSIONS.

	200 mg/m²		275 mg/m²	
	6 HR	24 HR	6 HR	24 HR
Peak plasma concentration (µM)	5.3	0.51	7.6	0.94
Area under curve (hr·mg/l)	24.6	10.6	54.7	13.5
Plasma disappearance, $t_{1/2}$ α (hr)	0.31	0.59	0.66	0.70
β (hr)	9.2	--	8.3	--
Mean residence time$_{inf}$ (hr)	9.5	15	10	19
Mean residence time$_{iv}$ (hr)	6.5	3	7	7
Apparent volume distribution$_{ss}$ (l)	90	125	61	269
Total clearance (ml/min)	232	592	144	637
Renal clearance (ml/min)	18	23	8.5	8.9
Urine excretion (% of dose)	6.6%	3.9%	5.2%	1.4%

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Didemnin B
T84-0302
UTHSC
July 1986

San Antonio Phase I Trial of Didemin B

Slide, A schedule for Didemnin B was a single dose every four weeks. The mode of administration was an infusion in 50 cc's in normal saline over 30 minutes. We started at 1/3 of 1/10 of the LD10.

Slide, 44 patients have been entered on study receiving 80 courses of drug. The median performance status has been 1. All patients had had prior treatment.

Slide, The type of tumors in patients treated with Didemnin B show a heavy predominance of non small cell lung cancer, renal cell cancer, colon, pancreas and sarcoma.

Slide, The dose escalation scheme and the accrual on each level is shown here. It has taken 11 dose escalation to reach the maximally tolerated dose. This can be attributed to the fact that we had to start at 1/3 of 1/10 of the LD10.

Slide, Dose limiting toxicity for Didemnin B has been nausea and vomiting This slide details the incidence of nausea and vomiting at each of the dose levels. As one can see here the number of patients with nausea and vomiting increased with the increasing doses of the drug. At a dose of 3.47 mg/m^2 4 out of 4 patients developed the nausea and vomiting. The one patient at 4.51 mg/m^2 had nausea and vomiting which lasted three weeks to spite of antimetics.

Slide We have attempted to see if antimetics could increase the amount of drug which could be given. As shown on this slide in four patients receiving 3.47 mg/m^2 without prophylactic antimetics all four developed grade 2 to 3 nausea and vomiting. In the five patients receiving 3.47 mg/m^2 after prophalactic antimetics (with Reglan, Decadron, and Benadryl) only one of the five developed nausea and vomiting. Therefore we feel one can elimiate some of the nausea and vomiting at 3.47 mg/m^2.

Slide, The other toxicity we noted was an elevation in liver function tests. As noted here with increasing doses of drug the percent of patients with SGPT elevations increased. These elevations occurred from day 3 to 26 and resolved without sequalae.

Slide, Elevations SGOT were also noted with a similar pattern.

Slide, Elevations in bilirubin were also noted with a similar pattern. One of the patients had 3.47 mg/m^2 developed a bilirubin of 3.6.

Slide, The other toxicities noted sporadically with Didemnin B included diarrhea in two patients, increased prothrombin time in two patients, shortness of breath in 5 patients and a chills diaphoresis flushing and a hypotensive episode very much like an anaphylactoid episode in one patient. These last two syndromes of the shortness of breath and of anaphylactoid reactions may be attributable to the cremaphor the patients received. It should be noted that the one patient who developed an anaphylactoid reaction was later pretreated with the taxol regimen and for two subsequent courses the patient had no problems with the drug.

Slide, We found no significant effects in hemoglobin, white count, granulocytes lymphocytes platelets and no eye toxicity was noted with careful ophthalmologic exams pre and post Didemnin B.

Slide, The slide details the patients receiving multiple courses of Didemnin B to try to answer the question as to whether chronic toxicity has been seen. One patient has received 12 courses of Didemin B over a year. This patient had pancreatic cancer and has been stable now for that period time. Other indications of chronic toxicity have been seen in these limited studies.

Slide, No responses have been seen to date.

Slide, In conclusion the dose limiting toxicity of Didemin B is nausea and vomiting. The recommended dose for Phase II studies is 2.67 mg/m^2 without prophylactic antiemetics and 3.47 mg/m^2 with prophylactic antiemetics.

Slide, We studied the pharmacokinetics of Didemnin B. Three assays were attempted. These included a bioassay, an HPLC assay which was not sensitive enough and finally a radioimmunoassay.

Slide, For the radioimmunoassay antibodies to Didemnin C conjugate were raised in rabbits. The sensitivity of this assay is down to 20 picogram per milliliter.

Slide, Here we see the standard curve for Didemnin B by the radioimmunoassay. It has a good wide range of sensitivities.

Slide, Since this assay has just been perfected we only have one patient in which we have run the entire pharmacokinetic spectrum. Even with this sensitive assay levels are only detectable only out to forty minutes after infusion. In this one patient receiving 2.05 mg/m^2 the half life was 10.5 minutes with a volume of distribution at 761 mls and a clearance of 357 mls per minute. Obviously, more data is needed on this, but the half life appears shorter than expected.

Slide, Of course we were interested in peak plasma concentrations to try to model other in vitro work with Didemnin B. We do have this information available on 15 patients. As noted in this slide the peak concentration is essentially undetectable for the first 10 patients. For patients 11-15 levels have ranged from 1,100 to 16,000 picrogram per ml.

Slide, Several ancillary studies have been performed with Didemnin B. These have included effects on lymphocytes, effects on calmodulin and screening in the human tumor cloning system.

Slide, We need to recall that Didemnin B has been looked at as an immunosuppressive agent by Montgomery and Zukoski. They felt it inhibits lymphocyte protein synthesis. It inhibits concanavalan A induced lymphocyte blastogenesis and it inhibits graft versus host disease in mice. They intend to use the drug as a transplant drug for possibly limiting rejection of cardiac transplants.

Slide, Effects of Didemnin B have been studied on lymphocytes in patients. We have studied only 3 patients with no difference in the number of T or B cells after Didemnin B therapy. Functional studies do indicate depressed responsiveness of lymphocytes to PHA and concanavalan A after Didemnin B administration.

Slide, There are some other interesting findings for Didemnin B in relationship to blood elements. In vitro 30% of Didemnin moves from the plasma to the cells within 5 minutes, therefore we will be running the pharmokinetics on the red cells which could act as a compartment and perhaps a slow release form for Didemnin B. Secondly we have performed relative uptake studies in vitro and find the lymphocytes and monocytes concentrate the drug 400 times over what the red cell concentrates the drug. We have found no specificity of uptake by lymphocyte subsets and we found no binding to calmodulin by Didemnin B. We must keep in mind that cyclosporin binds to calmodulin and this is thought to be a mechanisim of action of the drug against lymphocytes.

Slide, We have explored the activity of Didemnin B in the human tumor cloning system. This has also been explored in a limited way in Tucson. Combined results for 4 tumor types are shown here. One likes to see at least a 20% in vitro response rate to say whether drug will have activity or not in vitro. At the concentrations which appear to be clinically achievable, the compound has essentially no activity in non-small cell, lung, renal and ovarian cancer. However, with small numbers (6 total patients) 4 out of the 6 in this combined series of patients with sarcoma have responded. Therefore, the only hints we have for Phase II studies with Didemnin B at this time would be to try it in patients with sarcoma.

San Antonio Phase I Trial of Didemnin B

1) Schedule: Single dose every 4 weeks
2) Mode of administration: Infusion in 150 cc normal saline over 30 minutes
3) Start at 1/3 of 1/10 of LD_{10}

Patient Characteristics

Number of patients	44	(80 courses)
Men/women	35/9	
Age in years		
Median	62	
Range	(19-75)	
Median performance status	1	
Number of patients with prior treatment	44	
Chemotherapy	44	
RT and chemotherapy	28	

Types of Tumors in Patients Treated with Didemnin B

Lung	
Non-small cell	17
Small cell	1
Kidney	6
Colon	5
Pancreas	4
Sarcoma	4
Melanoma	1
Head and Neck	1
Lymphoma	1
Prostate	1
Breast	1
Endometrial	1
Gastric	1

Dose Escalation Scheme/Accrual

Dose (mg/m^2)	No. of Patients
0.14	3
0.28	3
0.42	3
0.63	4
0.94	4
1.22	5
1.58	6
2.05	3
2.67	6
3.47	8
4.51	1

Incidence of Moderate to Severe Nausea and Vomiting (without antiemetic pretreatment)

Dose (mg/m^2)	Number of Patients at Risk	Number of Patients with Grade 2 or 3[*] toxicity (%)
0.14	3	0 (0%)
0.28	3	1 (33%)
0.42	3	1 (33%)
0.63	4	0 (0%)
0.94	4	1 (25%)
1.22	5	1 (20%)
1.58	6	4 (67%)
2.05	3	1 (33%)
2.67	6	2 (33%)
3.47	4	4 (100%)
4.51	1	1[+] (100%)

[*] Defined as vomiting requiring parenteral antiemetics
[+] Lasted 3 weeks despite antiemetics

Incidence of Nausea and Vomiting (with and without prophylactic antiemetics*)

Dose (mg/m^2)	# of Patients	Status	Number with Grade 2-3 Toxicities (%)
3.47	4	Without antimetic	4 (100%)
3.47	5	With antimetic	1 (20%)

*Antiemetic regimen = Reglan (2 mg/kg IVB), Decadron (25 mg IVB),
Benadryl (50 IVB) pretherapy
= Reglan 1 mg/kg IVB, Benadryl 25 mg IVB Q 4 hr
x 2 post therapy

Incidence of SGPT Elevations

Dose (mg/m^2)	No. of Patients at Risk	Number of patients with Grade 2 or greater toxicity*
0.14	3	0
0.28	3	0
0.42	3	0
0.63	4	0
0.94	4	0
1.22	5	0
1.58	6	1 (day 12)
2.05	3	2 (days 18, 26)
2.67	6	2 (days 7, 13)
3.47	8	3 (days 3, 3, 8)
4.51	1	1 (day 5)

*> 51 (maximum = 154).

Incidence of SGOT Elevations

Dose (mg/m^2)	No. of Patients at Risk	No. of Patients with Grade 2 or greater toxicity *
0.14	3	1 (day 13)
0.28	3	2 (days 16, 22)
0.42	3	0
0.63	4	0
0.94	4	0
1.22	5	0
1.58	6	1 (day 7)
2.05	3	0
2.67	6	1 (day 8)
3.47	8	4 (days 2, 3, 5, 5)
4.51	1	1 (day 5)

*SGOT >61

Incidence of Bilirubin Elevations

Dose (mg/m^2)	No. of Patients at Risk	No. of Patients with Grade 2-3 Toxicity
0.14	3	0
0.28	3	1 (day 22)
0.42	3	0
0.63	4	0
0.94	4	0
1.22	5	0
1.58	6	0
2.05	3	0
2.67	6	1 (day 17)
3.47	8	2 (day 3, day 6)
4.51	1	0

*Bilirubin >2.1 (maximum = 3.6)

Other Toxicities Noted Sporadically (grade 2 or greater)

Toxicity	No. of Patients	(Dose mg/m^2)
Diarrhea	2	(0.28, 0.94)
Increased Protime (3-5 seconds)	2	(0.42, 0.63)
Increased Fibrin split products	1	(0.94)
Infiltration without sequelae	1	(0.28)
Shortness of breath	4	(0.14, 0.63, 0.94, 2.05)
Chills, diaphoresis, flushing, hypotension	1	(2.05)*

*Pretreated with "Taxol regimen" for 2 subsequent courses without problems (? cremophor reaction)

1) No significant effects on hemoglobin, WBC, granulocytes, or platelets.
2) Inconsistent decrease in lymphocytes.
3) No eye toxicity noted with careful ophthalmologic exams pre- and post-Didemnin B.

Patients Receiving Multiple Courses of Didemnin B
(? chronic toxicity)

Number of Courses	Number of Patients
1	31
2	8
3	5
4	1
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	1*

*No major toxicities noted in this patient at 0.28 mg/m^2
Stable disease pancreatic cancer for 1 year now

Responses

No Responses

Conclusions

1) Dose-limiting toxicity of Didemnin B = nausea and vomiting.
2) Recommended dose for phase II studies
   2.67 mg/m^2 without prophylatic antimetics
   3.47 mg/m^2 with prophylatic anti-emetics

Didemnin B Assays Attempted

1) Bioassay - B16 melanoma P388
2) HPLC - not sensitive enough
3) Radioimmunoassay -

RIA Methodology for Didemnin B

1) Didemnin A (possessing N-terminal proline) was conjugated to bovine serum albumin.
2) Conjugate was mixed with Ribi adjuvant and injected subcutaneously into New Zealand White rabbits at one month intervals.
3) Didemnin B radiolabeled
4) Sensitivity = 20 picograms/ml

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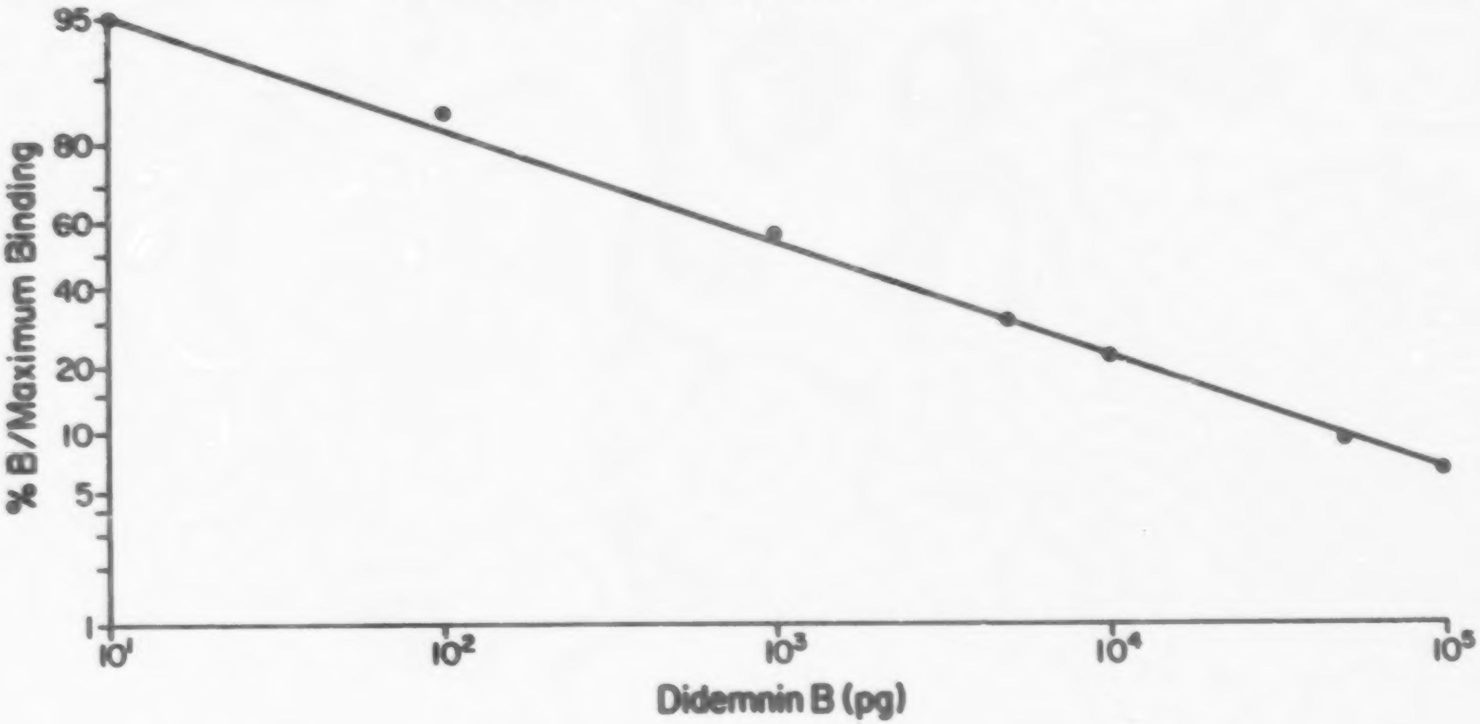
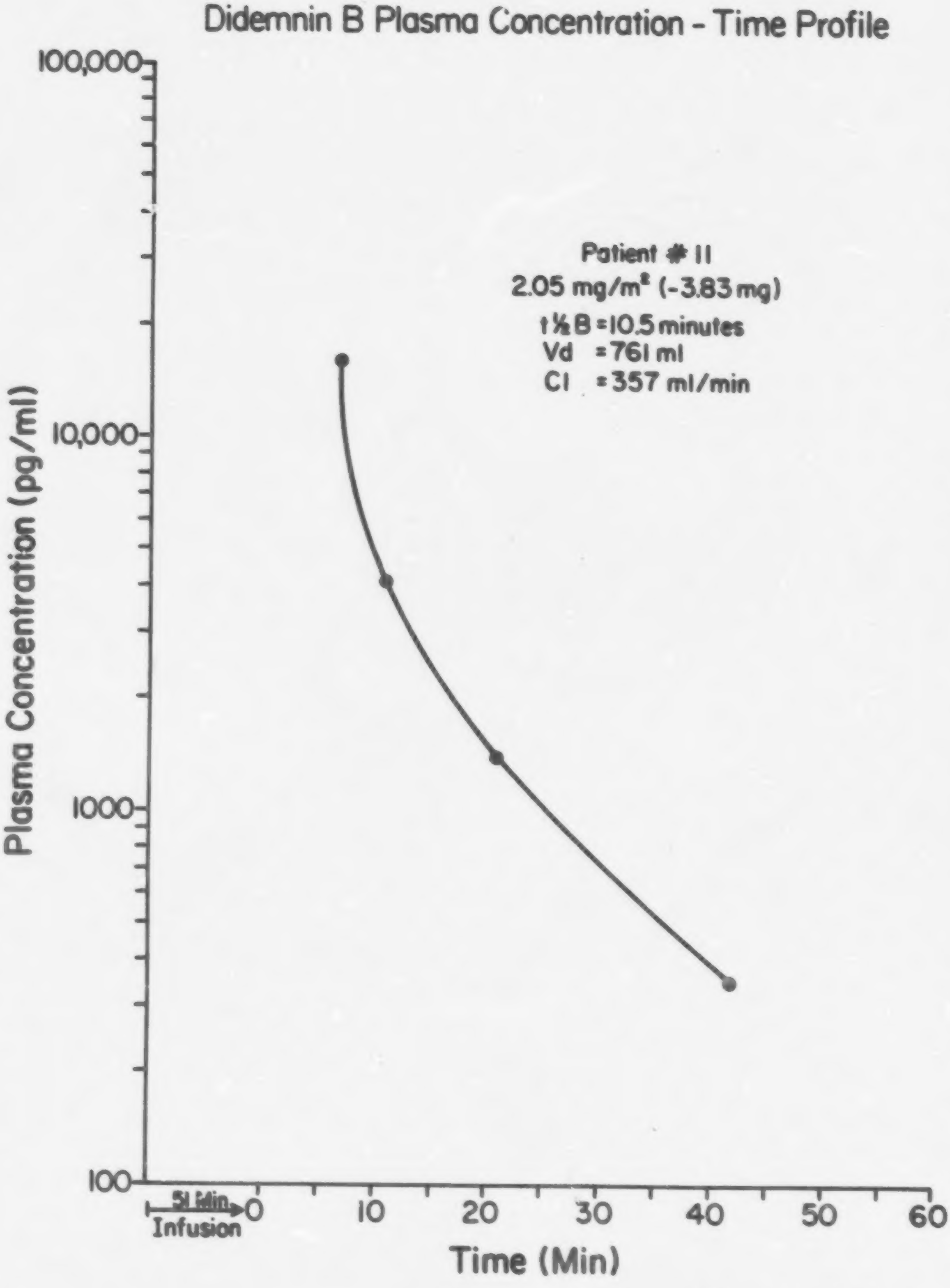
Didemnin B
T84-0302
UTHSC
July 1986

Standard Curve for Didemnin B Radioimmuno Assay




Didemnin B Plasma Concentration - Time Profile
Patient # 11
2.05 mg/m² (-3.83 mg)
t½B = 10.5 minutes
Vd = 761 ml
Cl = 357 ml/min
Plasma Concentration (pg/ml)
100,000
10,000
1000
100
51 Min
Infusion
0
10
20
30
40
50
60
Time (Min)

Didemnin B
Peak Plasma Concentrations

Pt #	Dose		BSA	Infusion Duration (min)	Peak Conc Pg/ml
	mg/m^2	mg			
1	0.28	0.47	1.67	30	–
2	0.42	0.66	1.56	30	–
3	0.63	1.0	1.65	30	–
4	0.94	1.38	1.47	31	1500 (5)$^+$
5	1.22	2.27	1.88	30	–
6	1.22	2.56	2.12	35	2000 (5)
7	1.58	2.72	1.7	32	–
8	1.58	2.75	1.76	37	–
9	1.58	3.16	2.0	30	–
10	2.05	3.98	1.94	30	–
11	2.05	3.83	1.87	51	16,000 (5)
12	2.05	4.12	2.01	45	1200 (5)
13	2.67	4.43	1.66	31	1100 (5)
14	3.47	5.48	1.58	38	3900 (0)
	(3.47)	(5.48)	*(1.58)	30	2000 (5)
15	4.51	7.94	1.76	35	6600 (5)

*Pharmacokinetics obtained on 2 separate occasions
$^+$Sample time-post infusion
–Non detectable

Ancillary Studies Performed with Didemnin B

1) Effects on lymphocytes
2) Effects on calmodulin
3) Screening in human tumor cloning system

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Didemnin B as an Immunosuppressive Agent*

---

1) Inhibits lymphocyte protein synthesis
2) Inhibits concanavalin A induced lymphocyte blastogenesis
3) Inhibits graft versus host disease in mice

---

*Montogomery and Zukowski, Transplantation 40: 49, 1985

Effects of Didemnin B on Lymphocytes in Patients
(n = 3)

---

1) No difference in number of T or B cells after Didemnin B therapy (24 hrs after)
2) Functional studies indicate decreased responsiveness of lymphocytes to PHA and Conconavalin A after Didemnin B administration

---

Findings for Didemnin B in Relation to Blood Elements
(*in vitro* findings)

---

1) *In vitro* 30% of Didemnin moves from plasma to cells within 5 minutes (run pharmacokinetics on whole blood)
2) Relative uptake *in vitro*:
    RBC = 1
    Lymphocytes and monocytes = 400
    Granulocytes = 28
3) No specificity of uptake by lymphocyte subsets (T vs B or T_4 vs T_8)
4) No binding to calmoditin (cyclosporin)

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Activity of Didemnin B in a Human Tumor Cloning System
(0.01 μg/ml - 1 hr exposure)

	# Responses/# Evaluable	
Tumor Type	Tucson	San Antonio
Non-small cell lung	0/2 (0%)	3/16 (19%)
Renal	0/1 (0%)	0/4 (0%)
Ovary	0/8 (0%)	0/3 (0%)
Sarcoma	2/3 (67%)	2/3 (67%)

Didemnin B
T84-0464
Vermont
July 1986

DIDEMNIN B

Daily x 5
University of Vermont/Vermont Regional Cancer Center

Thirty two patients have been treated with 38 courses of Didemnin B. Twelve dose levels have been tested. The schedule is a daily x 5 bolus intravenous administration.

Major toxicities have included nausea and vomiting as well as hepatotoxicity manifested by transient elevation of transaminases. At .54mg/m^2 and higher, nausea and vomiting is a significant problem requiring antiemetic therapy. At 1.6mg/m^2 daily x 5, four patients were treated and four patients had grade 2 - 3 nausea and vomiting.

One patient at 1.6mg/m^2 had an elevation of SGPT to 10 times the normal. This value returned to normal by day 20. No signs of chronic hepatotoxicity or clotting abnormalities have been noted.

To date, no drug related myelosuppression has been seen and no antitumor responses have been noted. It appears that the maximum tolerated dose will be defined by nausea and vomiting and hepatotoxicity.

DIDEMNIN B
(Daily X 5)

PATIENT CHARACTERISTICS

PATIENTS: 32		MEDIAN	RANGE
18 Male	Age	58	(33-72)
14 Female	Age	56	(27-75)
Performance Status (ECOG)		1	(0-2)

PRIOR THERAPY:

RADIATION THERAPY	3
CHEMOTHERAPY	15
CHEMOTHERAPY & RADIATION	9
NONE	5

TUMOR TYPES:

COLORECTAL	12
LUNG, NON-SMALL CELL	5
MELANOMA	3
BREAST	2
OVARIAN	2
RENAL CELL	2
SQUAMOUS CELL - H & N	2
PANCREAS	1
THYROID	1
LIPOSARCOMA	1
CHONDROSARCOMA	1

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Didemnin B
T84-0464
Vermont
July 1986

DIDEMNIN B
(Daily X 5)

DOSE ESCALATIONS

DOSE			NO. PATIENTS
0.03	mg/m^2	x 5	3
0.06	"	"	3
0.12	"	"	3
0.24	"	"	3
0.36	"	"	4
0.54	"	"	3
0.54	"	3	2
0.67	"	5	3
0.84	"	"	3
1.00	"	"	3
1.25	"	"	3
1.60	"	"	3
1.60	"	3	1
2.00	"	5	1

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Didemnin B
T84-0464
Vermont
July 1986

DIDEMNIN B
(Daily X 5)

HEMATOLOGIC TOXICITY

PATIENT	WBC X 10^3/ml PRE-THERAPY	WBC X 10^3/ml NADIR	WBC X 10^3/ml NADIR DAY	PLATELETS X 10^3/ml PRE-THERAPY	PLATELETS X 10^3/ml NADIR	PLATELETS X 10^3/ml NADIR DAY
1.25mg/m^2 d x 5						
L.P.	5.7	—	—	213	121	8
L.L.	5.3	—	—	302	—	—
A.B.	7.7	6.6	12	413	—	—
1.6mg/m^2 d x 5						
F.H.	10.9	9.5	17	469	393	7
A.S.	16.2	—	—	411	405	7
K.H.	7.4	—	—	390	369	8

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DIDEMNIN B
(Daily x 5)

Incidence of Moderate to Severe Nausea and Vomiting

Dose (mg/m^2) daily x 5	Number of patients treated	No. of patients with grade 2-3 toxicity
0.03	3	0
0.06	3	0
0.12	3	0
0.24	3	0
0.36	4	0
0.54	5	3
0.67	3	2
0.84	3	1
1.00	3	2
1.25	3	3
1.60	4	4
2.00	1	1

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DIDEMNIN B

(Daily x 5)

NAUSEA AND VOMITING

- Nausea and Vomiting escalates over 5 days
- Modified with anti-emetics
- Nausea and anorexia typically resolves by day 7 - 8

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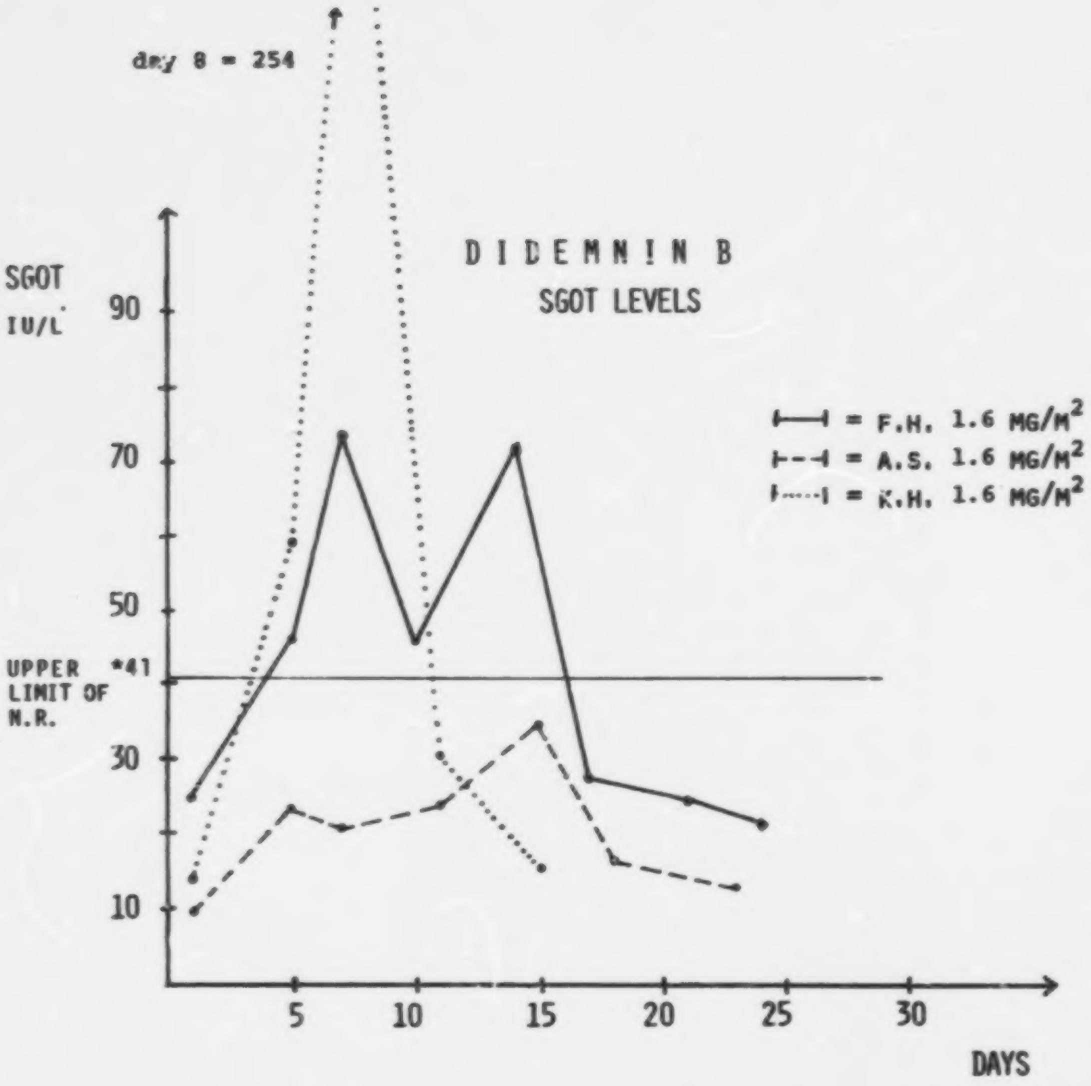
Didemnin B
T84-0464
Vermont
July 1985



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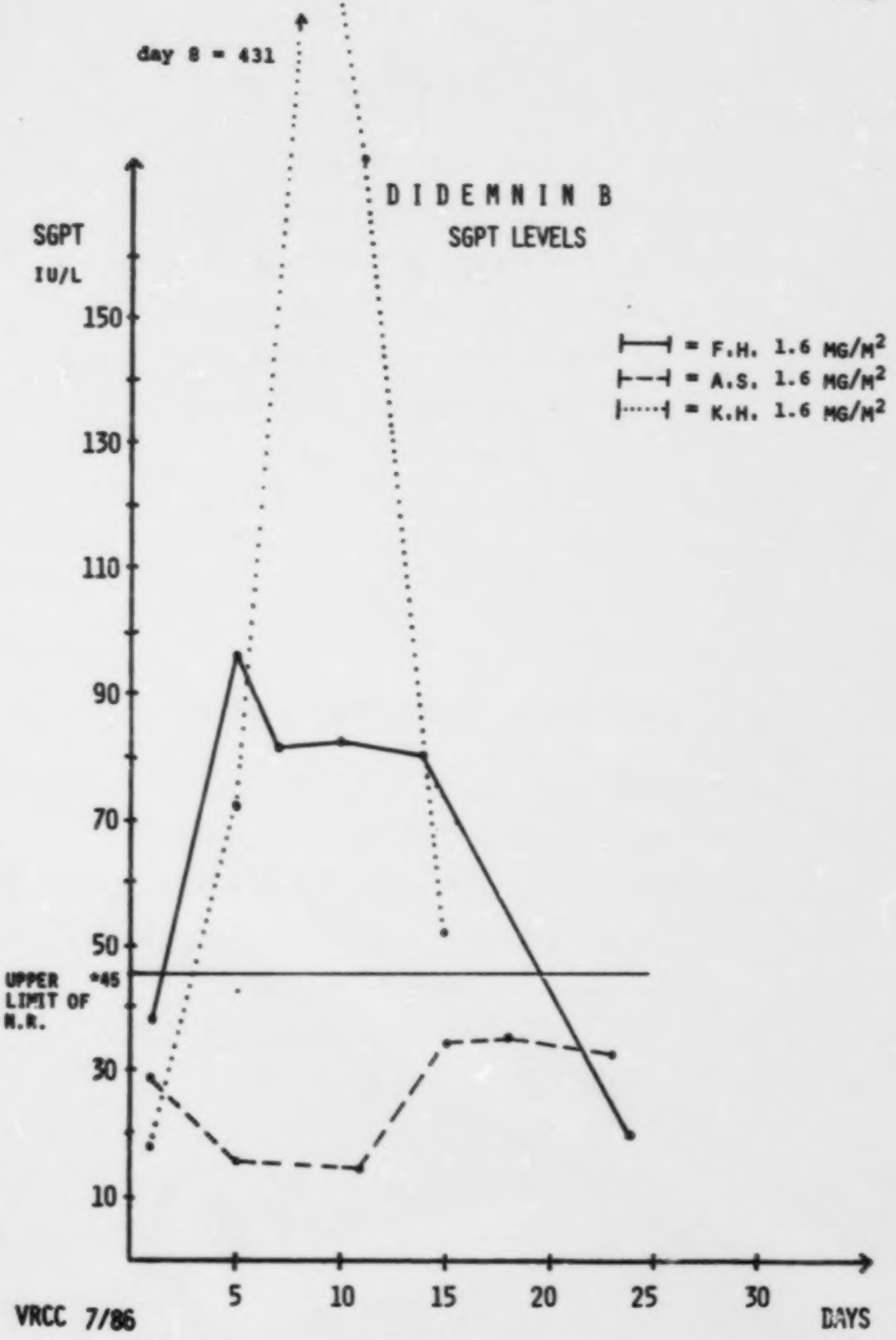
3/12

Didemnin B
T84-0464
Vermont
July 1986



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DIDEMNIN B

PLASMA SAMPLE PREPARATION

CYANO BOND ELUT EXTRACTION COLUMNS

SAMPLE DILUTED (1/3) WITH WATER/ACETONITRILE (80/20)

WASH WITH WATER AND DILUTE KOH (0.001N)

ELUTE DIDEMNIN B WITH METHANOL

CONCENTRATE AND DILUTE WITH MOBILE PHASE

PLASMA RECOVERY 85%

HPLC METHODOLOGY FOR DIDEMNIN B ANALYSIS

COLUMN: ALLTECH C 3 RSIL (250 x 4.5 MM)

ALLTECH TMS CARTRIDGE GUARD COLUMN (10 x 4.6 MM)

OPERATING TEMPERATURE: 58° C (CIRCULATING WATER JACKET)

MOBILE PHASE: ACETONITRILE/50MM KH_2PO_4 (57/43)
0.01% TRIETHYLAMINE

FLOW RATE: 1.0 ML/MIN DETECTION: 220 NM

DETECTION LIMIT: 5 NG

PATIENT'S DIDEMNIN B LEVEL

TIME (MIN)	AT 0.86 MG/M^2 (NG/ML)	AT 1.6 MG/M^2 (NG/ML)
10	56	103
20	15	DETECTED
30	-	DETECTED
40	NOT DETECTED	DETECTED

BILE FLOW (ML/HR/10 G LIVER
DIDEMNIN INITIAL CONC. (µG/ML)

TIME(MIN)	10	20	40	CONTROL (TMTX) (10 µG/ML)
0-30	0.48	0.57	0.30	0.84
30-60	0.43	0.54	0.32	0.84
60-90	0.33	0.33	0.22	0.71
90-120	0.17	0.20	0.10	0.68

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RBC UPTAKE OF DIDEMNIN B AT 37°
INITIAL CONC. WHOLE BLOOD 500 μG/ML

TIME (MIN)	CONC. (μG/ML)
10	34
20	60
120	80

SUMMARY

1. HPLC METHOD IS DEVELOPED, BUT INCREASE IN THE SENSITIVITY OF THE ASSAY IS NECESSARY FOR PRACTICAL USE
2. VERY LITTLE DIDEMNIN B IS RECOVERED FROM URINE AS PARENT COMPOUND
3. SIGNIFICANT REDUCTION IN BILE FLOW IN PERFUSED RAT LIVER MODEL
4. DIDEMNIN B IS TAKEN UP BY RBC

Pibenzimol
T84-0301
OSU
July 1986

PIBENZIMOL

We have reported our evaluation of Pibenzimol at previous meetings. We are currently studying only patients who have no evidence of glucose intolerance or pancreatitis, due to our observation of our initial studies that Pibenzimol caused dose related pancreatic toxicity including pancreatitis and hyperglycemia.

All patients are being carefully evaluated post treatment for pancreatic injury. Thirteen patients have been evaluated. The characteristics of these patients are shown on Slide 1. The primary site is shown on Slide 2. No patients with pancreatic cancer have been treated. Toxicity data is shown on Slide 3. It is to be noted that since we believe the pancreatic toxicity is cumulative, we tried to treat patients for at least two courses prior to escalation to the next dose level. Only minor toxicity has been seen including mild nausea and vomiting and mild creatinine elevation which may not be drug related. One patient just completed a course at 12 mgm/m^2. There was no side effects seen. We will continue to accrue patients at this dose level.

Pibenzimol
T84-0301
OSU
July 1986

PIBENZIMOL

CHARACTERISTICS OF 13 PATIENTS

AGE	RANGE	26 - 71
	MEDIAN	49.4

INITIAL PERFORMANCE STATUS

0	2
1	5
2	3
3	3
4	0

SEX	- MALE	10
	- FEMALE	3

PRIOR THERAPY

NONE	3
CHEMOTHERAPY	10
RADIATION THERAPY	5

PRIMARY SITE

BLADDER	1
HEAD/NECK	5
SKIN	2
LUNG	1
KIDNEY	1
GI TRACT	3

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PIBENZIMOL STUDY

ALL PATIENTS MUST HAVE NORMAL AMYLASE LEVELS AND GLUCOSE TOLERANCE TESTS PRIOR TO STUDY

TOXICITY

LEVEL 6 MGM/M^2 CIV x 5 DAYS

	0	1	2	3	4
NAUSEA AND VOMITING		7			
RENAL-CREATININE		1			

LEVEL 8.4 MGM/M^2 CIV x 5 DAYS

	0	1	2	3	4
NAUSEA AND VOMITING		2			

NO PANCREATIC TOXICITY SEEN. WILL TRY TO FINISH ACCRUAL AT 12 MGM/M^2 DOSE LEVEL WITH EACH PATIENT RECEIVING 2 OR MORE COURSES OF THERAPY.

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Pibenzimol
T84-0463
Mayo Clinic
July 1986

Phase I-II Study of Pibenzimol

Background

1. A fluorescent molecule which binds to double stranded DNA

2. Previously used exclusively as a stain for chromosomal analysis

3. Antitumor screen revealed activity against P388 and L1210 leukemias

4. Phase I study at OSU produced insulin requiring diabetes in four patients

5. Autopsy findings in one patient with pancreatic cancer suggested preferential retention of this fluorescent molecule in the primary and hepatic metastases

PHASE I-II STUDY OF PIBENZIMOL

Goals

1. To determine the maximum tolerated dose of Pibenzimol in patients treated for pancreatic cancer with this agent by continuous infusion over 120 hours
2. To assess preliminarily the therapeutic efficacy of Pibenzimol in patients with advanced pancreatic carcinoma
3. To determine the pharmacokinetic characteristics of Pibenzimol given by 120 hour infusion

PHASE I-II STUDY OF PIBENZIMOL

Eligibility Requirements

1. Histologic or cytologic confirmation of unresectable pancreatic adenocarcinoma
2. Adequate nutrition
3. Ambulatory (ECOG PS $\leq$ 2)
4. Serum creatinine $\leq$ 1.5
5. WBC $\geq$ 4000, HGB $\geq$ 10, PLT $\geq$ 130,000

Pibenzimol
T84-0463
Mayo Clinic
July 1986

PHASE I-II STUDY OF PIBENZIMOL

Contraindications

1. Recent surgery
2. Infection
3. Prior radiation > 30% marrow
4. Primary or metastatic CNS cancer
5. History of pancreatitis
6. Severe uncontrolled diabetes mellitus

PHASE I-II STUDY OF PIBENZIMOL

Methodology

1. A minimum of 3 patients treated at each level
2. Patients retreated at four weeks if disease stable or regressed and no toxicity seen
3. Hyperglycemia anticipated and not considered a dose limiting toxicity in this study

Pibenzimol
T84-0463
Mayo Clinic
July 1986

PHASE I-II STUDY OF PIBENZIMOL

Drug dose and schedule

Route: IV infusion - continuous

Schedule: daily x 5 days (120 hr)

Starting dose	mg/m^2/day
	6
Escalation 1	10
Escalation 2	12.5
Escalation 3	15
Escalation 4	18

PHASE I-II STUDY OF PIBENZIMOL

Dose mg/m^2/d	No. Pt. Treated
6	3
10	5
12.5	5
15	4
18	1

Four patients have received > one course

One patient has received four courses.

Pibenzimol
T84-0463
Mayo Clinic
July 1986

PHASE I-II STUDY OF PIBENZIMOL

Toxicity

1. No WBC < 3,500
2. No thrombocytopenia
3. No pancreatitis (clinical)
4. Nausea and vomiting - mild
5. One patient is requiring insulin to control drug induced diabetes

PHASE I-II STUDY OF PIBENZIMOL

Toxicity

Dose $mg/m^2/d$	Fasting Glucose > 150 mg/dL
6	0/3
10	0/5
12.5	2/5
15	3/4
18	1/1

PHASE I-II STUDY OF PIBENZIMOL

Conclusions

1. Pibenzimol given in this fashion is well tolerated
2. No evidence of anti-tumor effect
3. Hyperglycemia has been seen commonly at doses of ≥ 12.5 $mg/m^2/d$
4. Insulin dependent diabetes mellitus would be acceptable toxicity if anti-tumor effect is seen in metastatic pancreatic cancer

Merbarone
T85-0257
OSU
July 1986

MERBARONE

Merbarone, a barbiturate derivative with an unknown mechanism of antitumor activity, recently began Phase I trials at Ohio State University. Six patients have entered on study. Three at the initial dose level of 96 mgm/m^2 CIV x 5 days and three at 144 mgm/m^2 CIV x 5 days. This second dose level was reduced from the original proposed level due to the pharmokinetics to be discussed by Dr. Malspeis. The characteristics of the six patients entered on the study are shown on the next slide. The majority of patients have previously received therapy with either radiation and/or chemotherapy. All patients treated (Slide 3) had solid tumors refractory to standard therapy.

All three patients at the 96 mgm/m^2 level have received 2 courses of therapy while one of three patients at 144 mgm/m^2 have had two courses. There has been no hematologic toxicity. Toxicities that were seen include mild nausea and vomiting seen in 2 patients at 96 mgm/m^2 and one patient at 144 mgm/m^2 level. One patient had grade 2 renal toxicity. Although it was unclear that it was drug related since it occurred after the patient was placed on Indocin. Four patients developed significant phlebitis at the infusion site. This occurred on day 5 and day 6 at the first dose level, and on day 4 and day 3 at the 2nd dose level. The cause of the phlebitis is not known. We have attempted to prevent it by increasing the volume of solution the drug is dissolved in from 500 cc to 2000 cc. One patient who previously developed phlebitis received the second course without problems using the larger volume.

Merbarone
T85-0257
OSU
July 1986

PRIMARY SITE

- COLON	2
- HEAD/NECK	1
- KIDNEY	2
- OVARY	1

SIX PATIENTS ENTERED ON STUDY

- 3 AT 96 MGM/M^2 CIV X 5 DAYS
- 3 AT 144 MGM/M^2 CIV X 5 DAYS

CHARACTERISTICS OF 6 PATIENTS

AGE:		
RANGE	44 - 60	
MEDIAN	52.5	
INITIAL PERFORMANCE STATUS:		
1	1	(16.7%)
2	2	(33.3%)
3	3	(50.0%)
SEX:		
MALE	3	(50.0%)
FEMALE	3	(50.0%)
PRIOR THERAPY:		
NONE	1	(16.7%)
CHEMOTHERAPY	4	(66.7%)
RADIATION THERAPY	0	(0.0%)
CHEMOTHERAPY & RADIATION THERAPY	1	(16.7%)

TOXICITY

	96 MGM/M^2 GRADE				144 MGM/M^2 GRADE			
	1	2	3	4	1	2	3	4
NAUSEA AND VOMITING	1	1			1			
RENAL		1						
PHLEBITIS		2				2		

PHLEBITIS

- OCCURRED AT INFUSION SITE
- APPEARS TO BE CONCENTRATION DEPENDENT
- DID NOT OCCUR WHEN VOLUME OF INFUSION CHANGED FROM 500 CCS TO 2000 CCS

Merbarone
T85-0257
OSU
July 1986

MERBARONE

Pharmacology Report

THE OHIO STATE UNIVERSITY

INTRODUCTION

Clinical pharmacokinetic studies have been conducted in conjunction with the phase I evaluation of merbarone (NSC 336628, MB). The present report describes the plasma pharmacokinetics in 6 patients during and following the five day continuous infusion of the drug, the renal excretion in 5 of the patients and studies to characterize the human metabolites of MB.

METHODS

1. Analytical Method

A reversed-phase HPLC method was developed for the quantitation of plasma and urine levels of MB. The protein of plasma or urine (50 µl) was precipitated with 260 µl of MeOH-DMSO (85:15, v/v) containing 3´-F-merbarone (NSC 372106), the internal standard. After centrifugation, the supernatant (200 µl) was diluted with the mobile phase less methanol (300 µl) and 350 µl was injected on column. Chromatography was carried out on a 4 µm Nova-Pak C_{18} column (3.9 mm x 15 cm) using an isocratic mobile phase and detection at 306 nm. The mobile phase was ammonium acetate (0.1 M)-methanol-acetic acid-$MgSO_4 \cdot 7H_2O$ (2.08 M) (60:30:8:2, v/v/v/v) containing sodium dodecyl sulfate 0.001 M. Employing 50 µl of plasma, concentrations of merbarone in the range 0.050-5.00 µg/ml were quantitated. The method is summarized in Tables 1 and 2.

Calibration curves were prepared daily and all samples were assayed in duplicate. The peak area ratios of merbarone to the internal standard were plotted against the concentrations of merbarone (Fig. 1). The best fit straight line was determined by least squares regression using a weighting factor of 1/peak area ratio squared to calculate the slope, x-intercept and correlation coefficient. The concentration of merbarone in the unknown samples were calculated using the results of the regression analysis.

2. Clinical Pharmacokinetics

Three blood samples were obtained during each day of the continuous infusion and at frequent intervals during 60 hours following the end of the infusion. Each blood sample was immediately centrifuged at 1800 x g for 10 minutes and the separated plasma was stored at -20° until assayed.

Plasma concentrations of merbarone as a function of time were NONLIN computer fitted using weights of $1/y^2_{obs}$. Initial fitting attempts utilized both the one and two compartment open model systems with a continuous zero-order infusion input until the observed end of the infusion. At the second dose level (144 mg/m^2/day x 5), sampling and timing techniques were established which permitted appropriate fitting of the data. Plasma concentration-time profiles following the end of the infusion were distinctly biphasic and afforded excellent NONLIN fits to the two compartment open model system. In order to properly define the terminal log-linear phase, it was necessary to extend blood sampling to 60 hours or longer after the end of the infusion. Careful monitoring of the true beginning and end of the infusion was required in order to properly define the rapid disposition phase. Care was also needed to insure a uniform infusion rate, particularly near the end of the infusion on day 5. It was determined that these precautions were essential for the appropriate definition of the disposition phases.

At the first dose level (96 mg/m^2/day x 5), the total body plasma clearance, Cl_T, for each patient study was calculated from the geometric mean of the steady state plasma levels determined during days 2-5 of the infusion. At the second dose level, Cl_T values were obtained from the computer fit of each entire data set.

3. Metabolite Characterization

UV spectra during chromatography were obtained with a Hewlett-Packard model 1040M diode array detector. Spectra were scanned from 210 nm to 400 nm during each 160-200 msec of the chromatograms. Chromatography was conducted with the analytical mobile phase and Nova-Pak C_{18} columns.

Patiant plasma samples were prepared as described in the analytical method. The parent drug and metabolites were isolated from urine samples using a short C_{18} solid phase extraction column. The urine (5 ml) was passed through the column. After washing the column with water (15 ml), the compounds were eluted from the column with methanol (1 ml) and the solvent was removed under a stream of nitrogen. The residue was reconstituted in the mobile phase (500 µl) and an aliquot (25 µl) was injected on column.

RESULTS AND DISCUSSION

1. Clinical Pharmacokinetic Studies

The plasma pharmacokinetics are reported for 6 patients treated with 96-144 mg/m^2/day x 5 (dose levels 1 and 2) of merbarone. The plasma concentration-time profiles for the three patients at the second dose level are shown in Figs. 2-4. The curve drawn through the experimentally determined plasma concentrations is the computer fit to the data. During the course of the infusion, the steady state plasma concentrations for these patients were 7-9 µg/ml. Following the end of the infusion, the plasma levels exhibited biphasic decline.

The individual pharmacokinetic parameters at the second dose level are listed in Tables 3 and 4. Cl_T for the three patients ranged from 10.5 to 14.2 $ml/min/m^2$. Following the end of the infusion, the initial half-life range was 0.8 to 3.7 hr and the terminal half-life range was 9.2 to 14.6 hr.

The central compartment volume of distribution, V_1, was small ranging from 4.6% to 16.0% of body weight (Table 4). The steady state volume of distribution, Vd_{ss}, was also relatively small ranging from 15.2% to 23.5% of body weight. Even the total body volume of distribution, Vd_{β}, was relatively small corresponding to 22.4% to 48.2% of body weight. Thus, it appears that merbarone is not extensively bound to deep tissue sites.

The total body clearance for 9 courses of merbarone ranged from 8.0 to 14.4 $ml/min/m^2$ (Table 5). At dose level 1, each patient was treated with two courses of the drug and for a given patient, the total body clearance for the two courses was similar (Table 5). The observation that both the total body clearance and the volumes of distribution were small suggests that merbarone is highly bound to plasma proteins.

The urinary excretion of merbarone was determined for 7 days after the start of the infusion for 7 of 9 courses administered (Table 6). The mean urinary excretion of the parent drug was 1.9% of the dose with a range of 0.3% to 4.3%. Accordingly, renal clearance of the unchanged drug is a minor component of the total body clearance.

2. Metabolite Characterization

Liquid chromatographic and UV spectral evidence has been obtained for the presence of two major metabolites and at least one minor metabolite in the urine of patients treated with merbarone. The major metabolites are tentatively identified to be 4′-hydroxymerbarone and 2-oxo-2-desthiomerbarone (Fig. 5).

Chromatograms of patient urine obtained during the continuous infusion of merbarone injected directly on column showed the presence of two major metabolites. One of these elutes shortly after the solvent front where urinary constituents are present. Therefore, a solid phase extraction column was used for sample clean-up. A chromatogram employing 306 nm detection of patient urine collected on day 5 during the infusion after clean-up shows two metabolite peaks at 1.6 and 6.9 min (Fig. 6). Based upon their chromatographic retention times relative to that of merbarone (8.4 min), both metabolites appear to be more polar than the parent drug. The chromatogram when examined at greater sensitivity shows two peaks at 11.9 and 15.5 min which were not seen in chromatograms of urine collected prior to dosing (Fig. 7). A chromatogram of a reference sample of 2-oxo-2-desthiomerbarone exhibits a retention time similar to that of the metabolite in urine eluting at 6.9 min (Fig. 8). Since the UV absorption maximum in the mobile phase of 2-oxo-2-desthiomerbarone occurs at approximately 278 nm whereas that of merbarone is at 306 nm, the chromatograms determined with 306 nm detection do not adequately convey the relative concentations of metabolite and parent drug.

Three-dimensional chromatograms of the urine extract qualitatively afforded an understanding of the relative concentrations of the metabolites and the parent drug (Figs. 9-12). The concentrations of the major metabolites eluting at 1.6 min and 6.9 min greatly exceed that of the parent drug, which elutes at 8.4 min; the predominant metabolite is the former (Fig. 9). The concentration of the metabolite eluting at 6.9 min relative to that of merbarone eluting at 8.4 min is shown in Fig. 10. Truncation of the 6.9 min metabolite peak is required in order to view the merbarone peak, which appears to be homogeneous (Fig. 11). With truncation of the merbarone peak, the metabolite eluting at 11.9 min is seen, however, the compound eluting at 15.5 min is present at a concentration which is exceedingly low at this sensitivity (Fig. 12). The characteristic UV spectra determined at the apex of the chromatographic peaks of a reference sample of 2-oxo-2-desthiomerbarone and the urinary metabolite eluting at 6.9 min proved to be similar (Fig. 13).

In order to further compare the chromatographic retention and UV spectra of the major urinary metabolites with reference samples of 4′-hydroxymerbarone and 2-oxo-2-desthiomerbarone, other columns and mobile phases were explored for prolonging the retention, however, significant tailing occurred. Accordingly, chromatography was carried out using two Nova-Pak C_{18} columns in tandem and the analytical mobile phase.

Chromatograms obtained with this system using 306 nm detection of similarly prepared extracts of urine collected prior to dosing and during day 5 of the infusion of merbarone are shown in Figs. 14 and 15, respectively. The major metabolite peak eluted at 3.3 min (Fig. 15). At increased sensitivity, the longer retention of the parent drug and other metabolites are readily seen (Fig. 16). The retention of the principal metabolite eluting at 3.3 min (Fig. 17) and that of a reference sample of 4′-hydroxymerbarone (Fig. 18) were similar. The retention of the major metabolite eluting at 12.9 min (Fig. 19) was similar to that of 2-oxo-2-desthiomerbarone (Fig. 20). Using the two columns in tandem, merbarone was found to elute at approximately 16 min (Fig. 21). Three-dimensional chromatograms of drug-free urine (Figs. 22 and 23) and urine collected on day 5 during dosing (Figs. 24-26) demonstrate, as before, the urinary concentrations of the two major and the minor metabolite relative to that of merbarone. Despite the presence of urinary constituents under the metabolite peak eluting at 3.3 min, the UV spectrum of the metabolite appears to be similar to that of 4′-hydroxymerbarone (Fig. 27).

The metabolite levels in the plasma were very low. Chromatograms determined at 306 nm of drug-free plasma, plasma obtained during day 5 of the continuous infusion and of a reference sample of merbarone are shown in Figs. 28, 29 and 30, respectively. A three-dimensional chromatogram from 1.4 to 18 min of a sample of patient plasma (50 µl) obtained before dosing exhibits a small peak at about 3 min and is otherwise free of interfering components (Fig. 31). A similar chromatogram of plasma obtained on day 5 during dosing shows the parent drug at 8.4 min and only an exceedingly small amount of the metabolite, 2-oxo-2-desthiomerbarone at 6.9 min (Fig. 32). The major urinary metabolite, 4′-hydroxymerbarone, is either absent or present in a trace amount. Upon increasing the sensitivity and truncating the merbarone peak, the 2-oxo metabolite peak can be viewed (Fig. 33). Examination of the 2-oxo metabolite peak relative to that of merbarone in the day 5 plasma of the three patients treated with 144 mg/m^2/day x 5 demonstrates the very low levels of this metabolite which are present in plasma (Figs. 34-36).

The UV spectrum determined at the apex of the chromatographic peak of merbarone in the plasma was similar to that of a reference sample (Fig. 37). The concentration of the 2-oxo metabolite in plasma was so low that a satisfactory UV spectrum could not be obtained using a 50 µl sample. Therefore, merbarone and the 2-oxo metabolite were isolated from 1 ml of plasma using a C_{18} solid phase extraction column and chromatographed. By averaging the spectra of the metabolite peak, it was possible to demonstrate the similarity of the UV spectra of the metabolite and a reference sample of 2-oxo-2-desthiomerbarone (Fig. 38).

Thus, LC and UV spectral evidence has been obtained that 4′-hydroxymerbarone and 2-oxo-2-desthiomerbarone are major urinary metabolites of merbarone. The concentrations of these metabolites in urine are much greater than that of the parent drug. In addition, at least one minor metabolite was seen in patient urine. Only very low levels of the 2-oxo metabolite were seen in patient plasma. The renal clearance of both major metabolites is greater than that of merbarone.

PHARMACOKINETIC AND METABOLIC SUMMARY

1. The total body plasma clearance, Cl_T, of merbarone during the 5 day continuous infusion of 96 mg/m^2/day and 144 mg/m^2/day ranged from 8.0 to 14.4 ml/min/m^2 for 6 patients.

2. Following the end of the infusion, the decline of the plasma levels, monitored to 60 hr after the 144 mg/m^2/day x 5 dose, appeared to be biphasic with $t_{1/2\alpha}$ ranging from 0.8 to 3.7 hr and $t_{1/2\beta}$ ranging from 9.2 to 14.6 hr.

3. Merbarone is not highly bound to the tissues with Vd_{ss} ranging from 5.6 to 8.7 L/m^2 (15 to 24 % of body weight).

4. Elimination is primarily non-renal. The renal excretion was approximately 2% of the dose and ranged from 0.3 to 4.3% in the 6 patients. The very low renal excretion is probably due to the high degree of plasma protein binding.

5. The total body clearance of merbarone in man is considerably smaller than reported for the dog. Therefore, the steady state plasma levels observed in man were higher than expected.

6. Chromatograms of patient urine show the presence of two major metabolites. The concentrations of these metabolites in urine are substantially greater than that of the parent drug.

7. The metabolites exhibit LC retention times and UV spectra similar to 4′-hydroxymerbarone and 2-oxo-2-desthiomerbarone.

TABLE 1

PLASMA SAMPLE PREPARATION FOR THE ASSAY OF MERBARONE

Plasma:	50 µl
Internal Standard:	3'-F-Merbarone (NSC 372106); 0.1 µg in Methanol-DMSO, 260 µl
Protein Precipitation:	Methanol-DMSO (85:15, v/v), 260 µl
Centrifugation:	12,000 x g, 10 min
Supernatant:	200 µl
Diluent:	Ammonium Acetate (0.1 M)-Acetic Acid (0.4 M)-$MgSO_4 \cdot 7H_2O$ (2.08 M) (90:8:2, v/v/v) containing Sodium Dodecyl Sulfate 0.001 M, 300 µl
Volume Injected:	350 µl

TABLE 2

HPLC ANALYSIS OF MERBARONE IN PLASMA

LC:	Isocratic
Column:	Nova-Pak C_{18}, 4 µm (3.9 mm x 15 cm)
Pre-column:	Guard-Pak, C_{18}
Mobile Phase:	Ammonium Acetate (0.1M)-Methanol-Acetic Acid (0.4 M)-$MgSO_4 \cdot 7H_2O$ (2.08 M) (60:30:8:2, v/v/v/v) containing Sodium Dodecyl Sulfate 0.001 M
Flow Rate:	1.0 ml/min
Detector:	306 nm, 0.005 AUFS
Lowest Conc. on Std. Curve:	50 ng/ml

TABLE 3

MERBARONE HALF-LIVES, MEAN RESIDENCE TIMES, TOTAL BODY CLEARANCE AND PERCENT OF THE AREA CORRESPONDING TO THE PHASES UNDER THE PLASMA CONCENTRATION-TIME CURVE (N=3)

Patient	Patient I.D. No.	Dose (mg/m^2/d)	$t_{1/2\alpha}$ (hr)	$t_{1/2\beta}$ (hr)	I.V. BOLUS MRT (hr)	CL_T (ml/min/m^2)	α-Phase AUC,%	β-Phase AUC,%
J.B.	900-29-7144	144	3.71	14.55	10.23	14.16	68.81	31.19
R.F.	900-31-9070	144	0.95	10.14	7.31	14.42	55.14	44.86
H.S.	900-32-9251	144	0.77	9.16	6.95	10.46	35.24	64.76

TABLE 4

MERBARONE VOLUME PHARMACOKINETIC PARAMETERS AND AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE OF PATIENTS (N = 3)

Patient	Patient I.D. No.	Dose (mg/m^2)	V_1 (L/m^2)	V_2 (L/m^2)	Vd_{ss} (L/m^2)	Vd_β (L/m^2)	$AUC_{0\to\infty}$ (μg.hr/ml)	Cp_{ss} (μg/ml)
J.B.	900-29-7144	144	5.92	2.76	8.69	17.82	847.7	7.03
R.F.	900-31-9070	144	1.99	4.34	6.33	12.65	832.3	7.03
H.S.	900-32-9251	144	1.71	3.91	5.62	8.30	1147.1	9.07

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TABLE 5

MERBARONE STEADY STATE PHARMACOKINETICS

Patient	Course	Dose ($mg/m^2/d$)	Cp_{ss} ($\mu g/ml$)	Cl_T ($ml/min/m^2$)
D.B.	1	96	6.28[a]	11.01
D.B.	2	96	5.30[a]	12.05
D.S.	1	96	8.31[a]	8.01
D.S.	2	96	7.33[a]	9.01
N.V.	1	96	6.60[a]	10.19
N.V.	2	96	7.11[a]	9.19
J.B.	1	144	7.03[b]	14.16
R.F.	1	144	7.03[b]	14.42
H.S.	1	144	9.07[b]	10.46

[a] Geometric mean Cp_{ss} calculated from days 2-5 steady state plasma levels

[b] Calculated from NONLIN fit of steady state and post-infusion plasma levels

TABLE 6

URINARY EXCRETION OF MERBARONE

Patient	Course	Dose (mg/m^2/d)	Dose (mg)	Aex (mg)	$\frac{Aex}{Dose}$ x 100 (%)
D.S.	1	96	801.5	22.1	2.76
D.S.	2	96	801.5	34.4	4.29
N.V.	1	96	825.0	12.0	1.45
N.V.	2	96	816.0	2.4	0.29
J.B.	1	144	1525.0	42.9	2.81
R.F.	1	144	1000.0	5.6	0.56
H.S.	1	144	1210.0	10.9[a]	0.90[a]
				Mean	1.87
				Range	0.29-4.29

[a] Missing one void collection during day 3.

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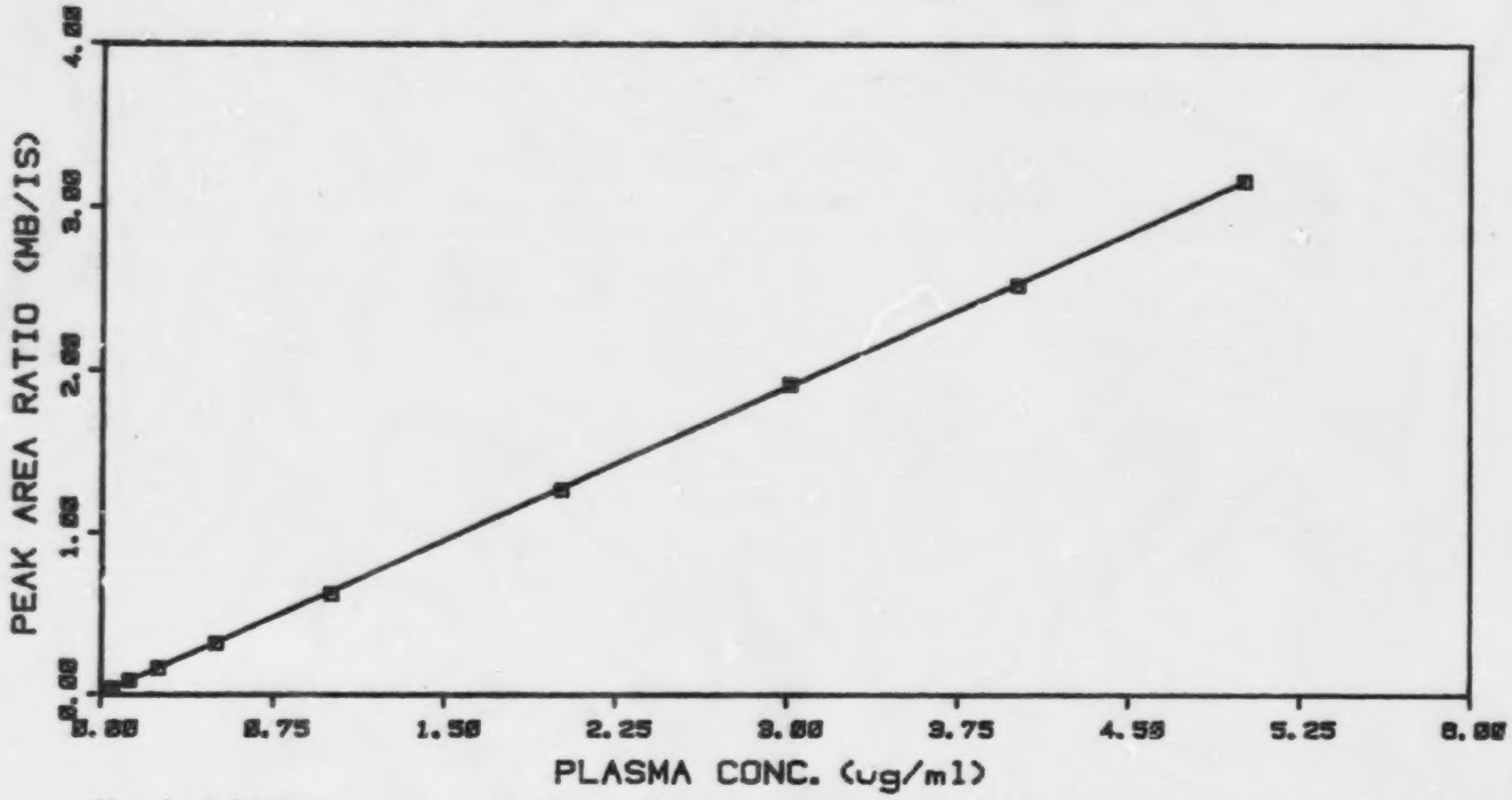

Fig. 1 Calibration curve for merbarone in patient plasma. Slope = 0.6268, intercept = 0.004167, r = 0.9999.

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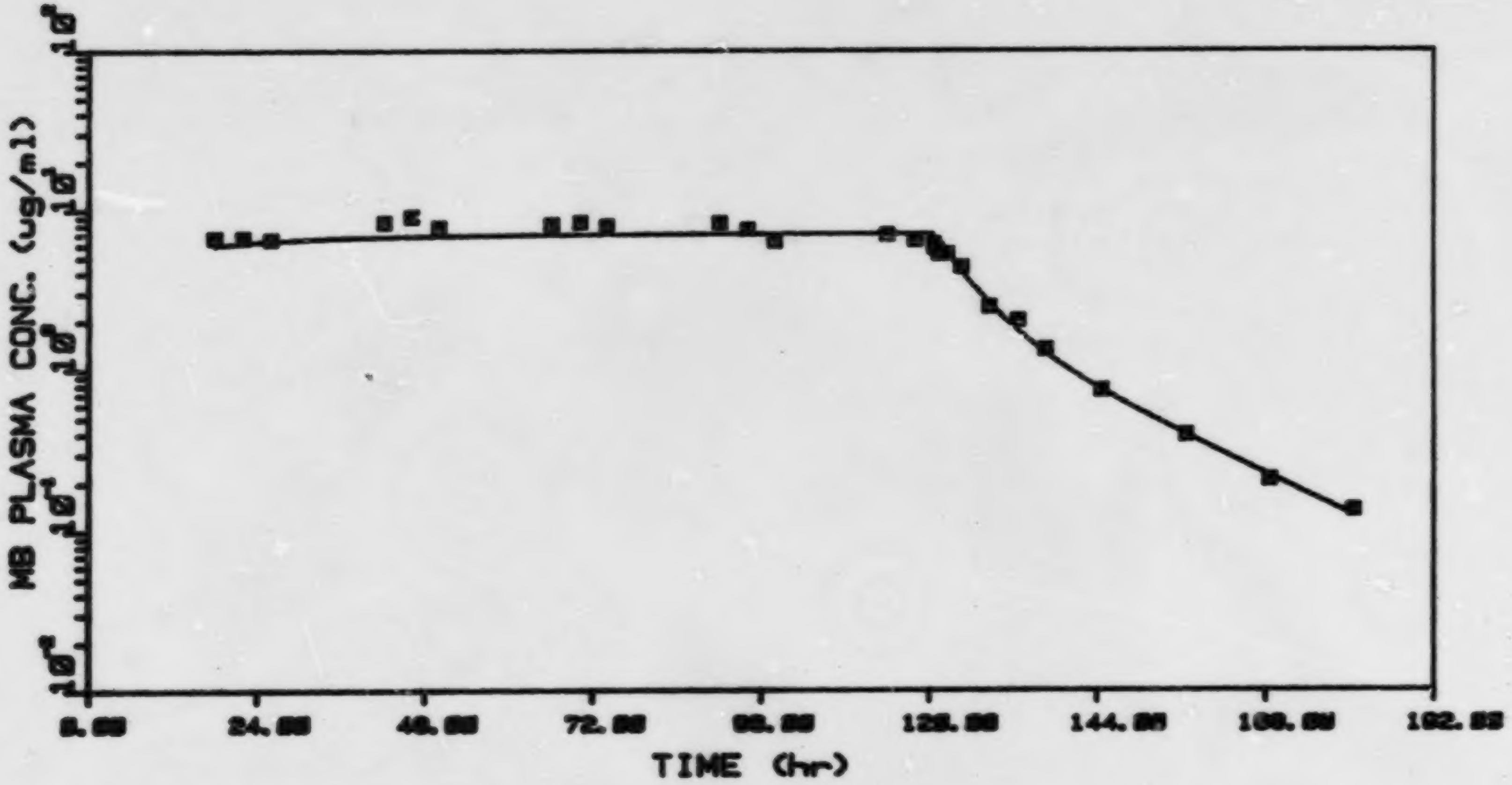

PATIENT J. B.
DOSE = 144 MG/M2/D × 5 MERBARONE
CLEARANCE = 14.16 ML/MIN/M2

Fig. 2

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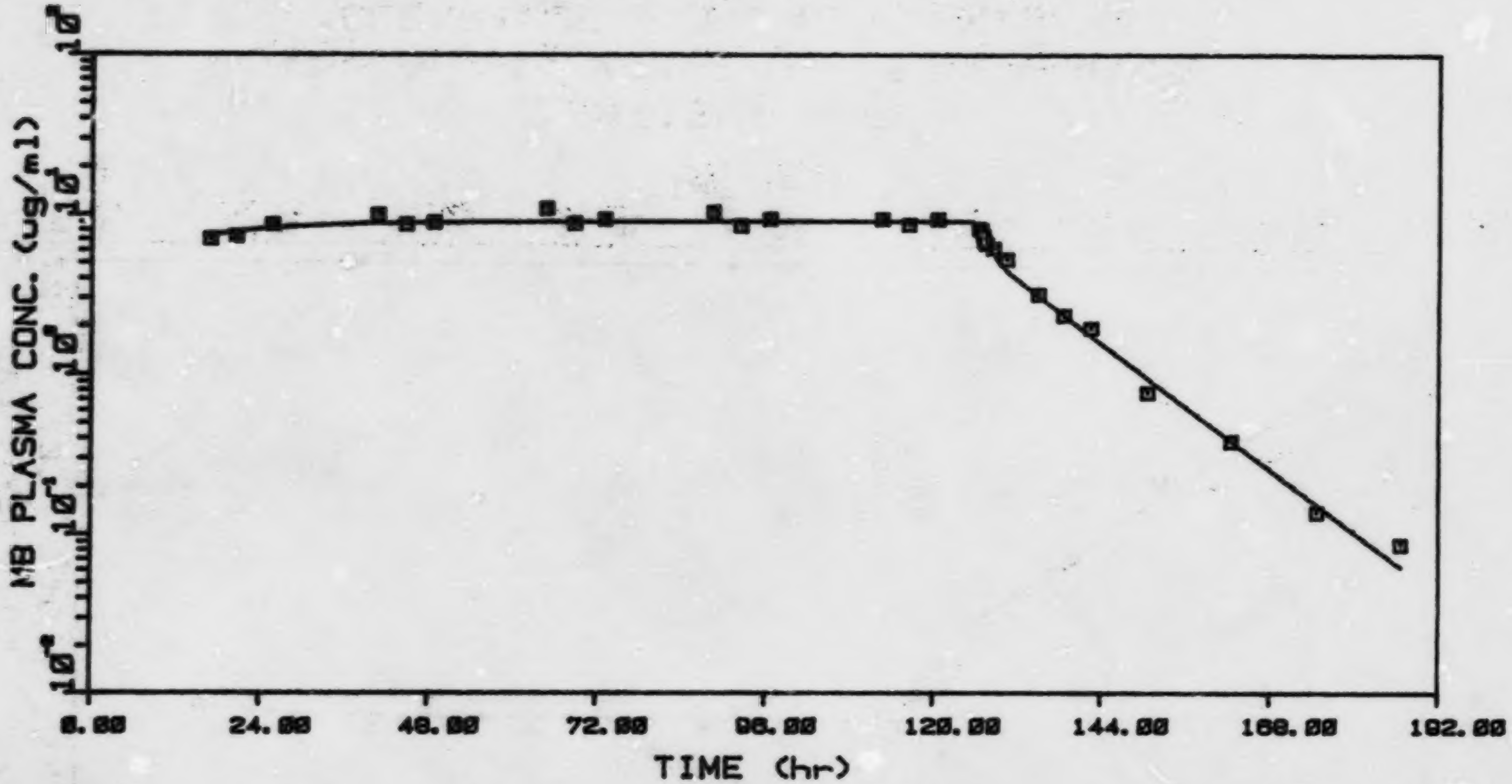

PATIENT H. S.
DOSE = 144 MG/M2/D × 5 MERBARONE
CLEARANCE = 10.47 ML/MIN/M2

Fig. 3

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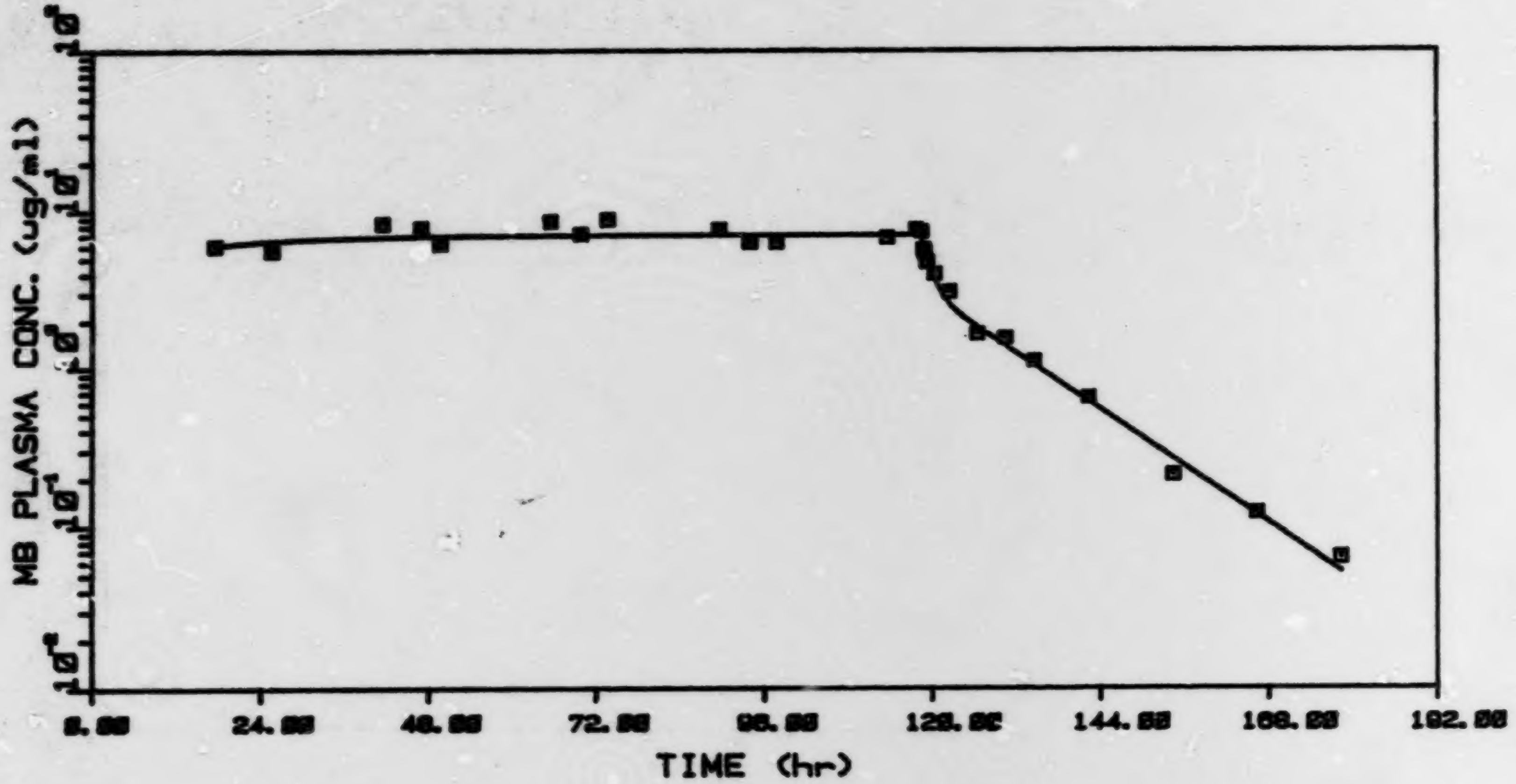

PATIENT R. F.
DOSE = 144 MG/M2/D × 5 MERBARONE
CLEARANCE = 14.42 ML/MIN/M2

Fig. 4

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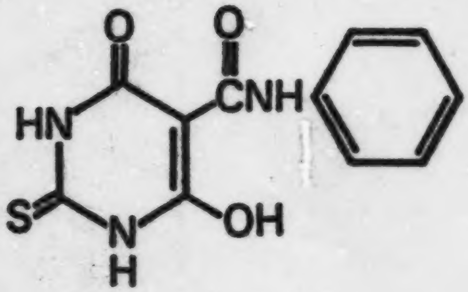
STRUCTURES OF MERBARONE AND ITS METABOLITES IN MAN



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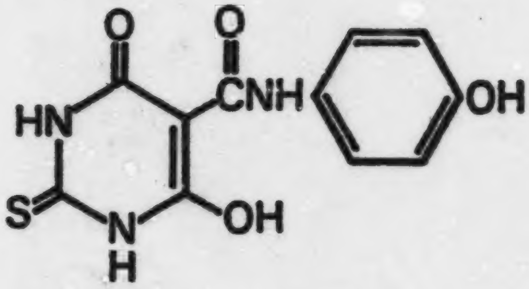
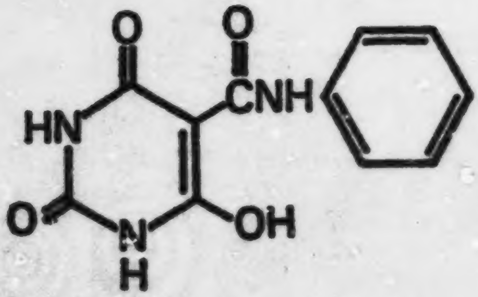


4'-HYDROXYMERBARONE



2-OXO-2-DESTHIOMERBARONE

Fig. 5

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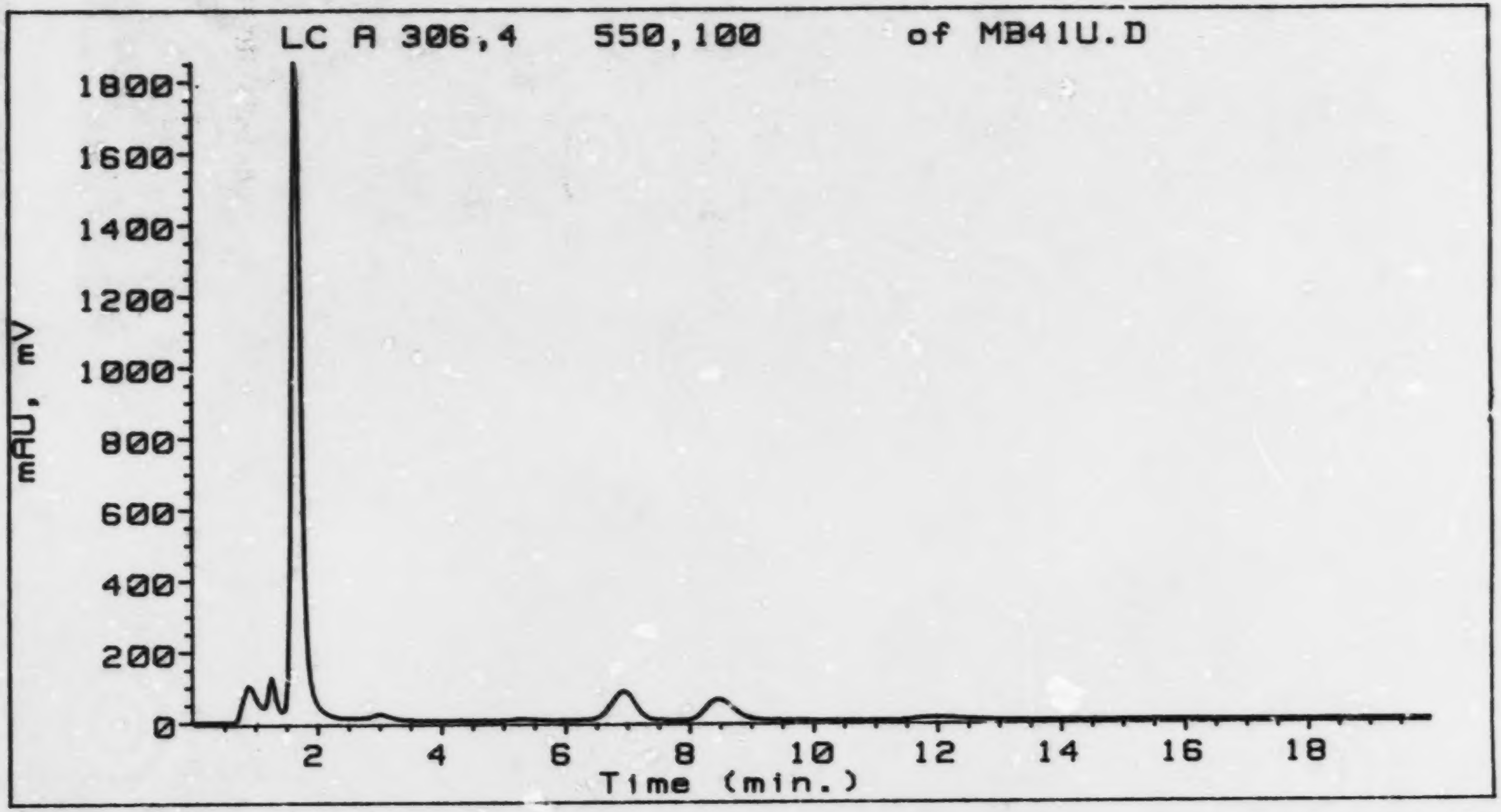

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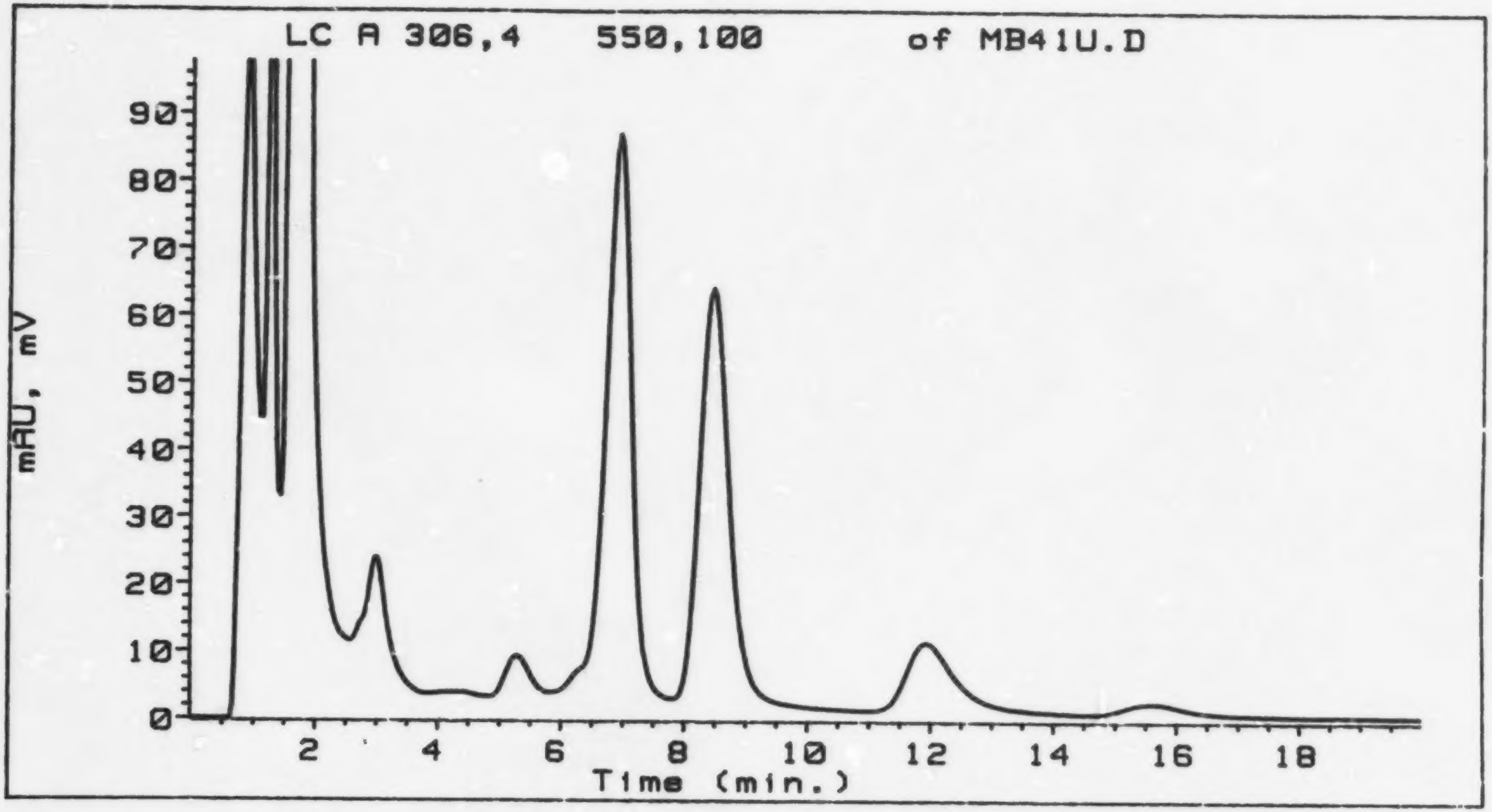

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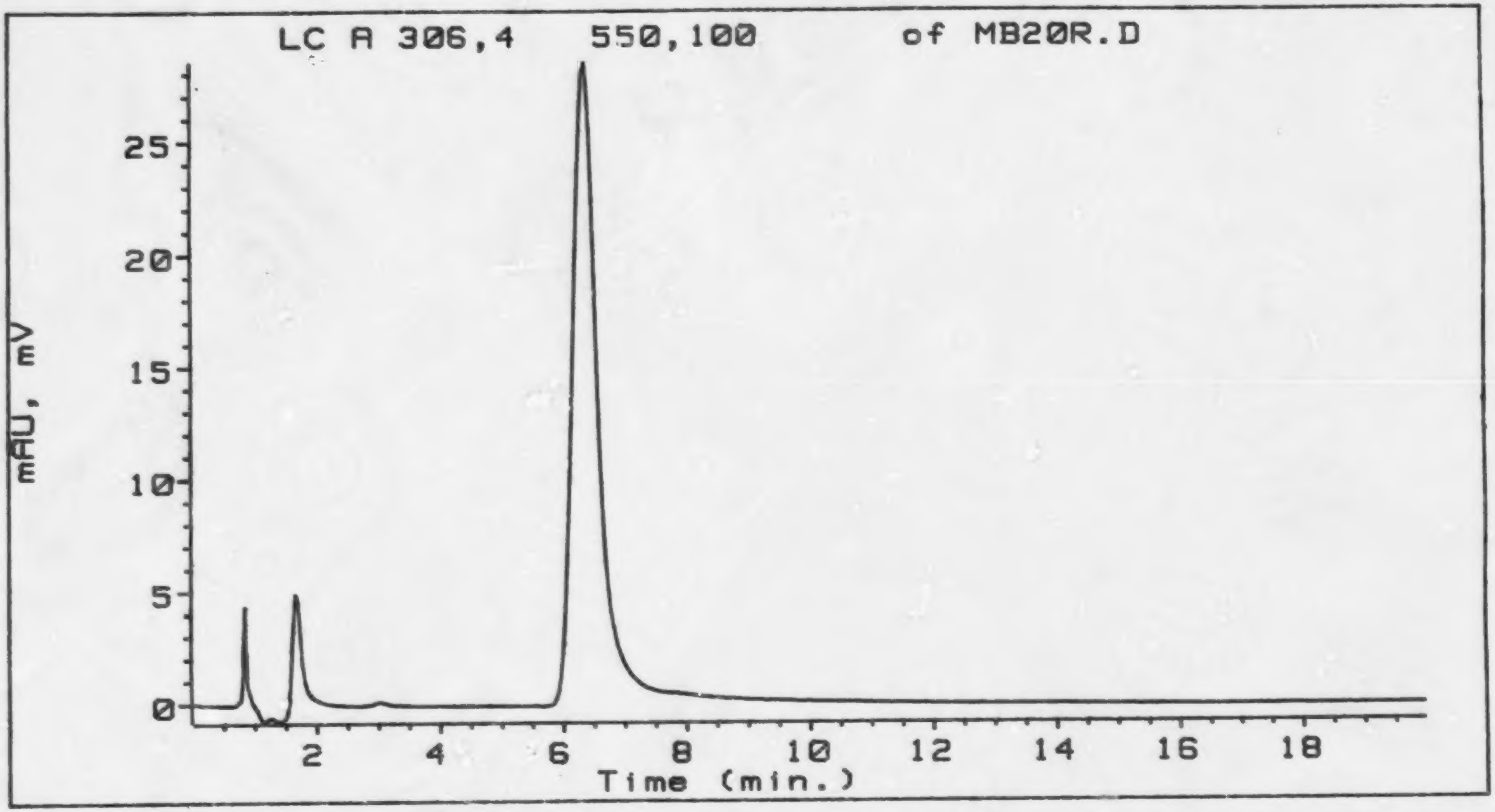

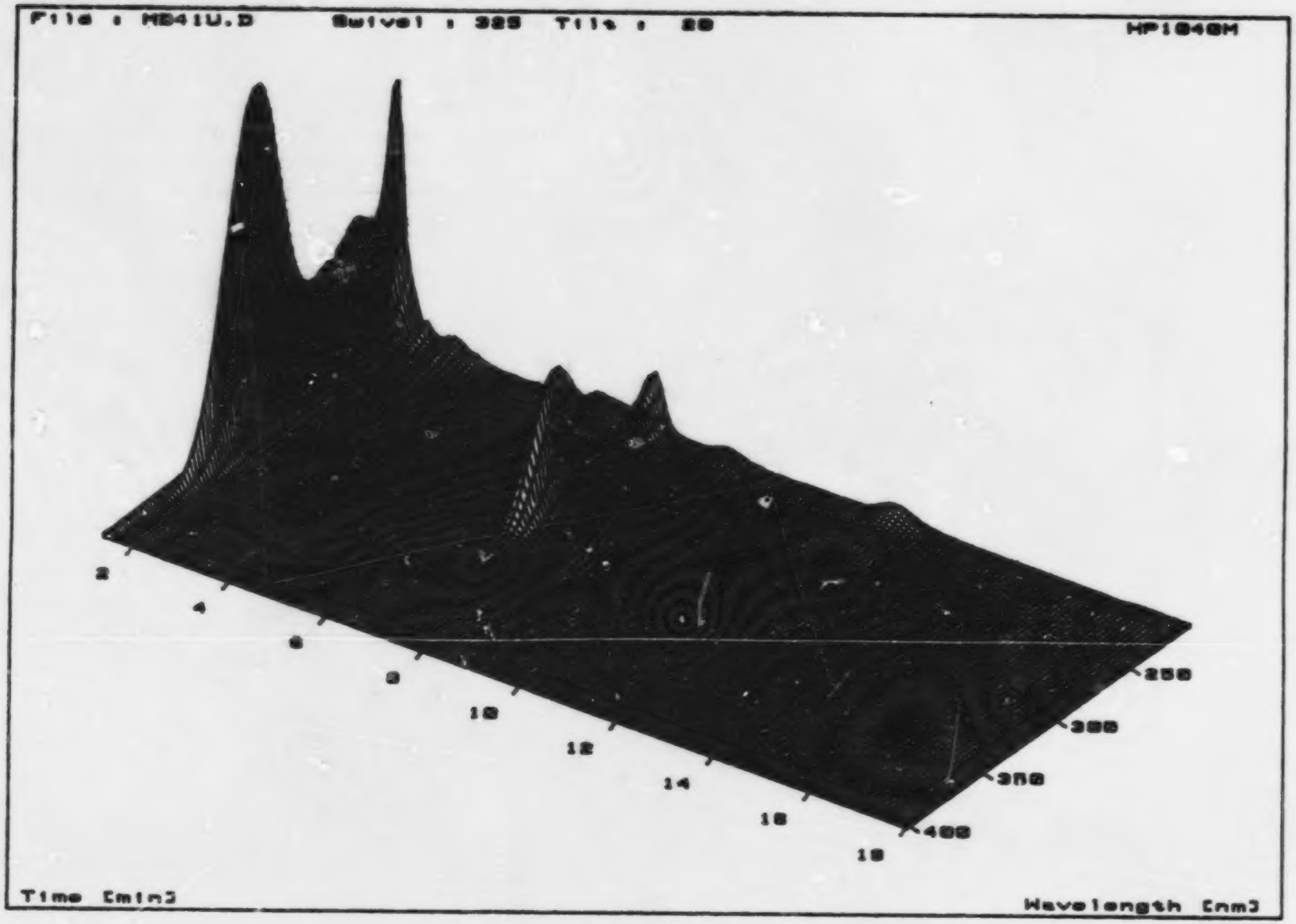

Fig. 9 Three-dimensional chromatogram from 1.5 to 18 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/dav x 5 illustrating that the relative concentrations of the metabolites eluting at 1.6 and 6.9 min are substantially in excess of the parent drug eluting at 8.4 min.

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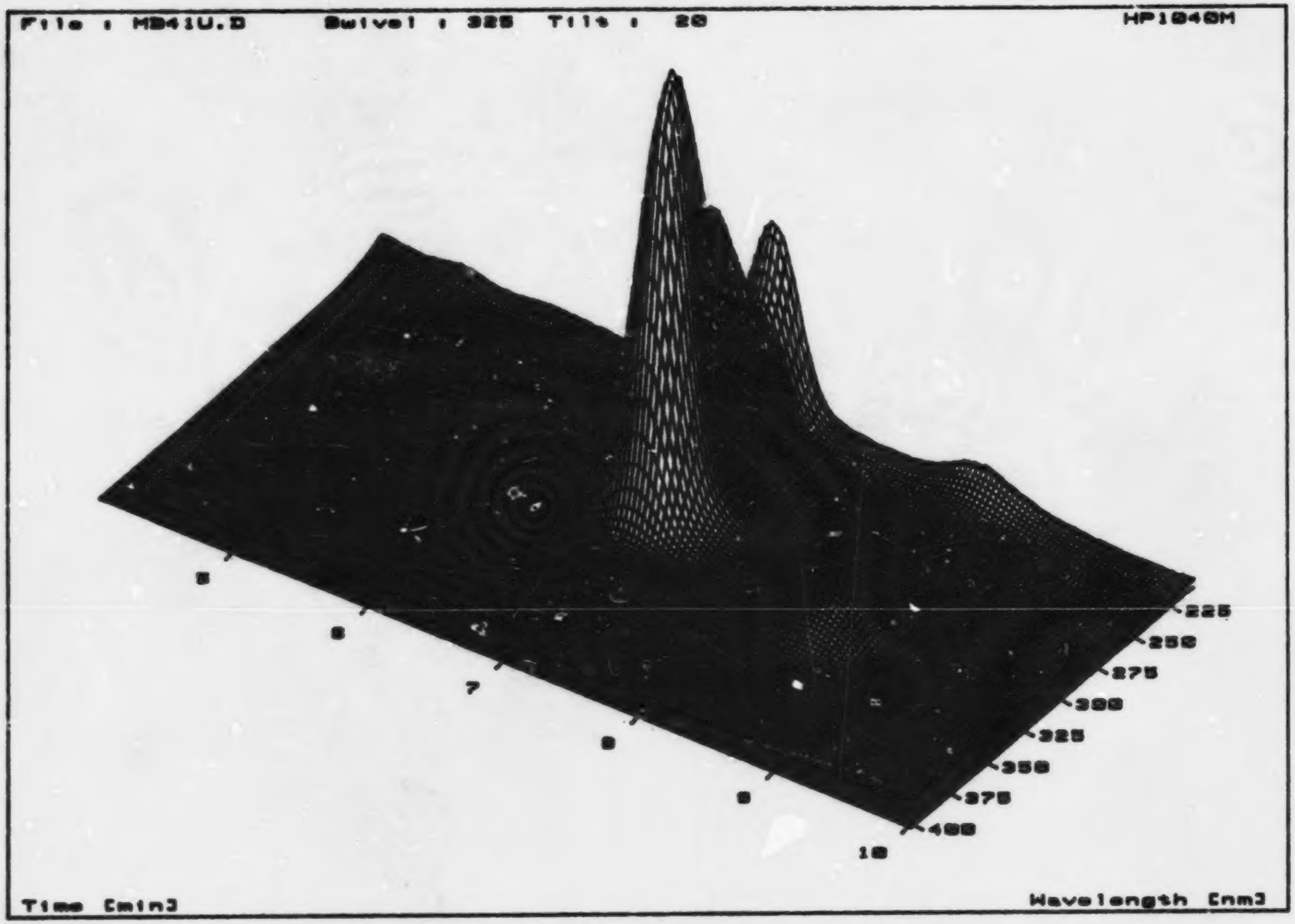

Fig. 10 Three-dimensional chromatogram from 4 to 10 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/day x 5 showing the concentration of the metabolite eluting at 6.9 min (2-oxo-2-desthiomerbarone) relative to that of merbarone which elutes at 8.4 min.

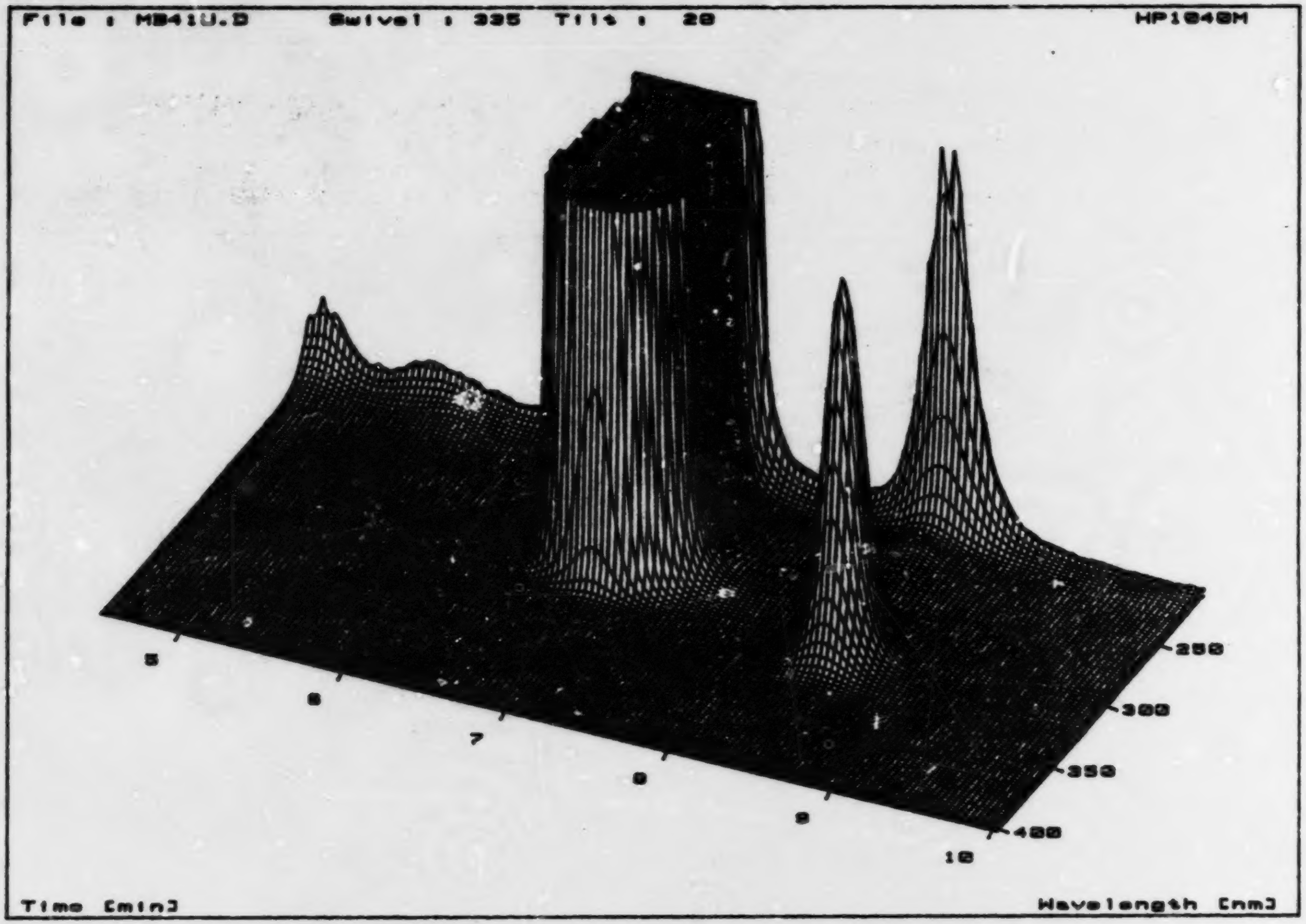

Fig. 11 Three-dimensional chromatogram from 4.5 to 10 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/day x 5. The metabolite peak (6.9 min) is truncated and the merbarone peak (8.4 min) is apparently homogeneous.

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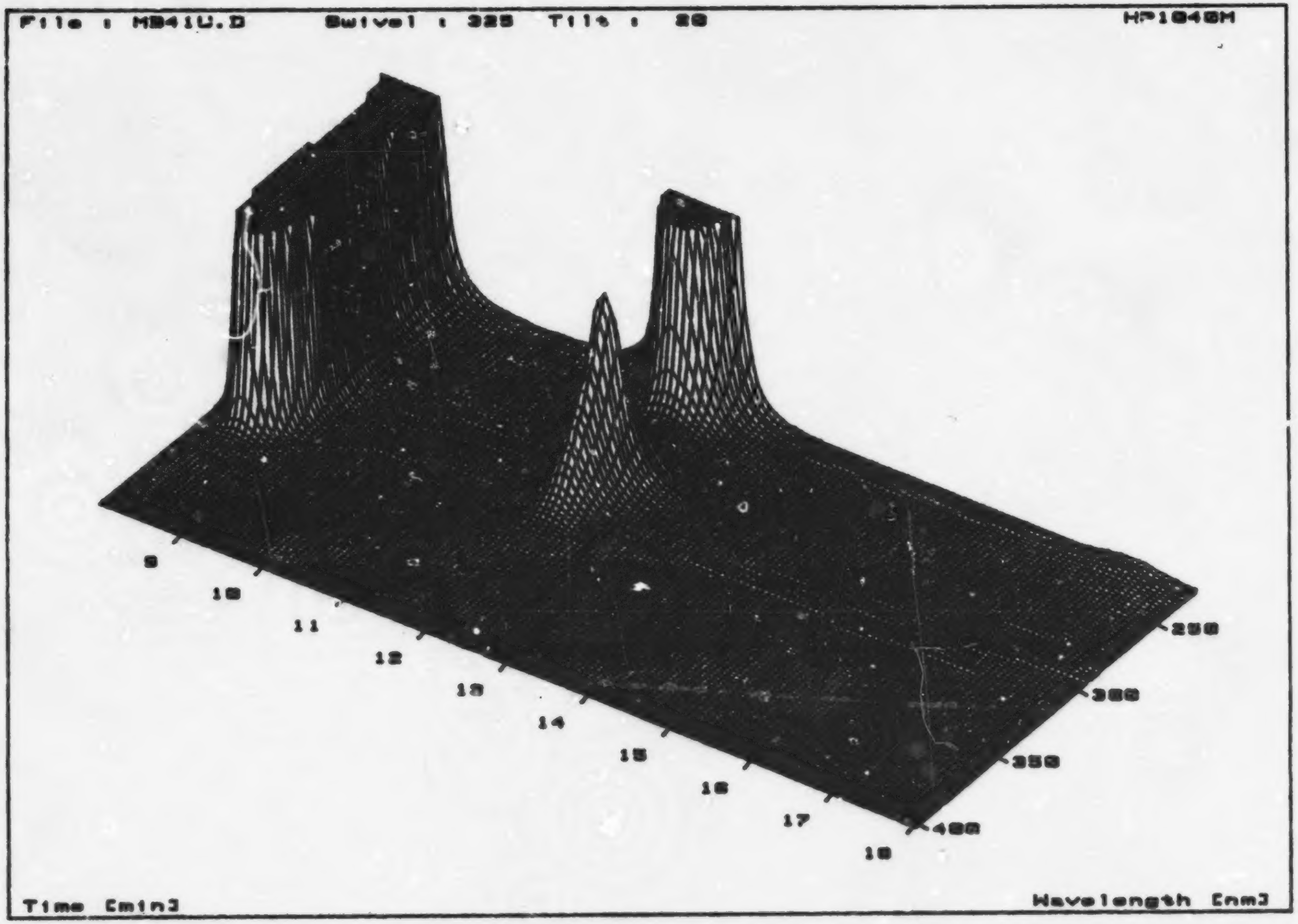
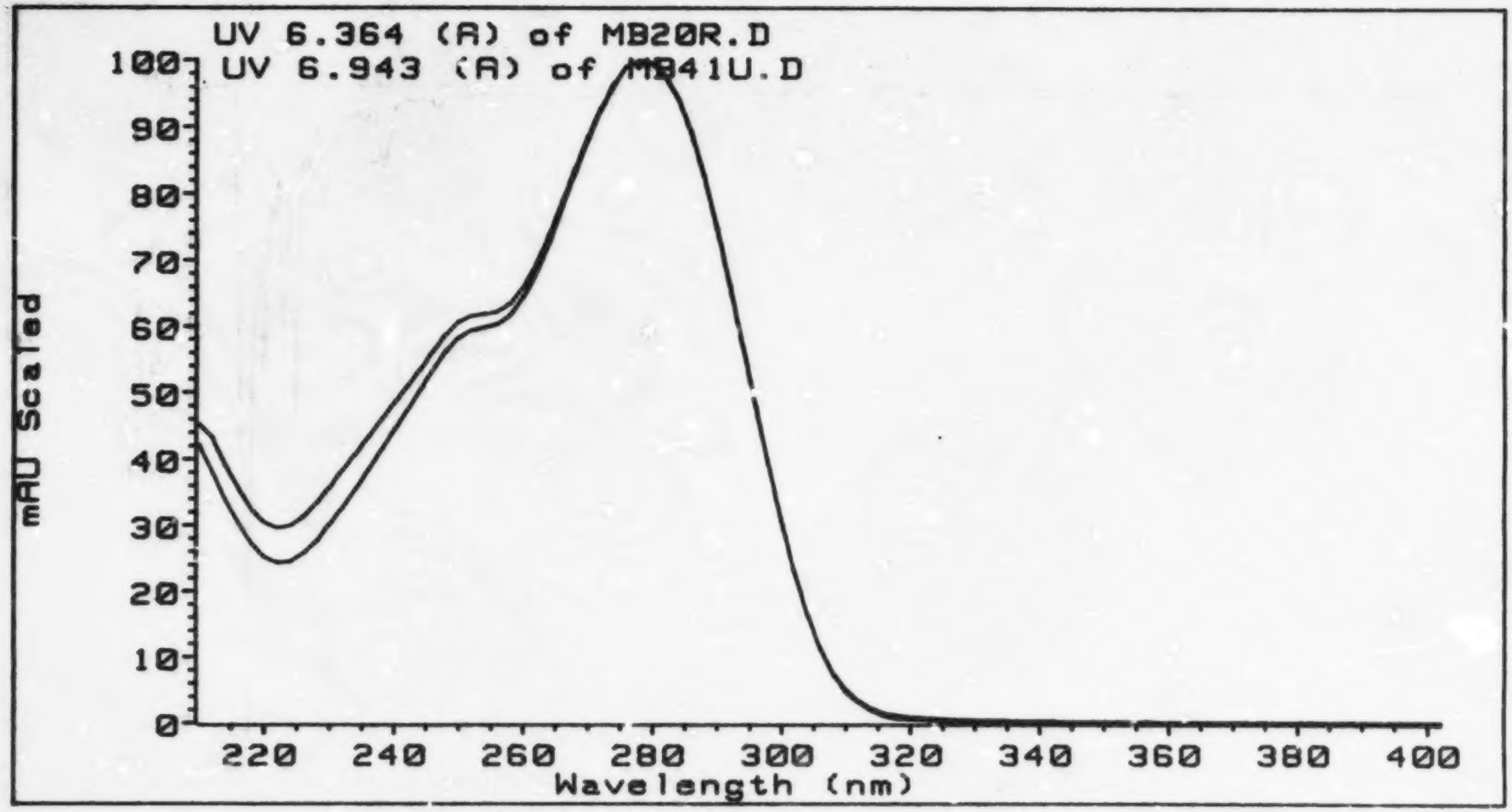


Fig. 12 Three-dimensional chromatogram from 8 to 18 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/day x 5. The merbarone peak (8.4 min) is truncated and the compound eluting at 11.9 min is a minor metabolite.



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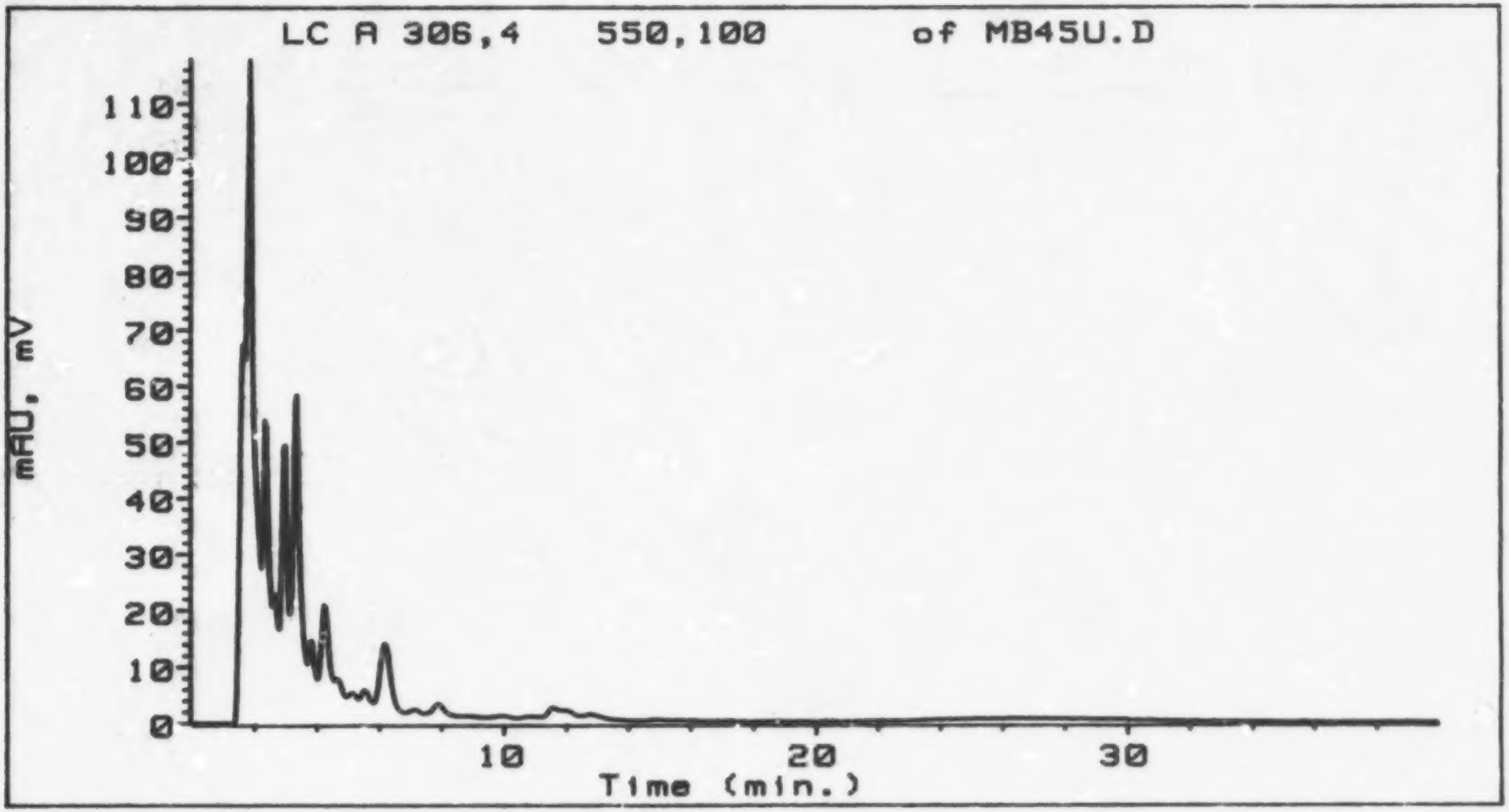

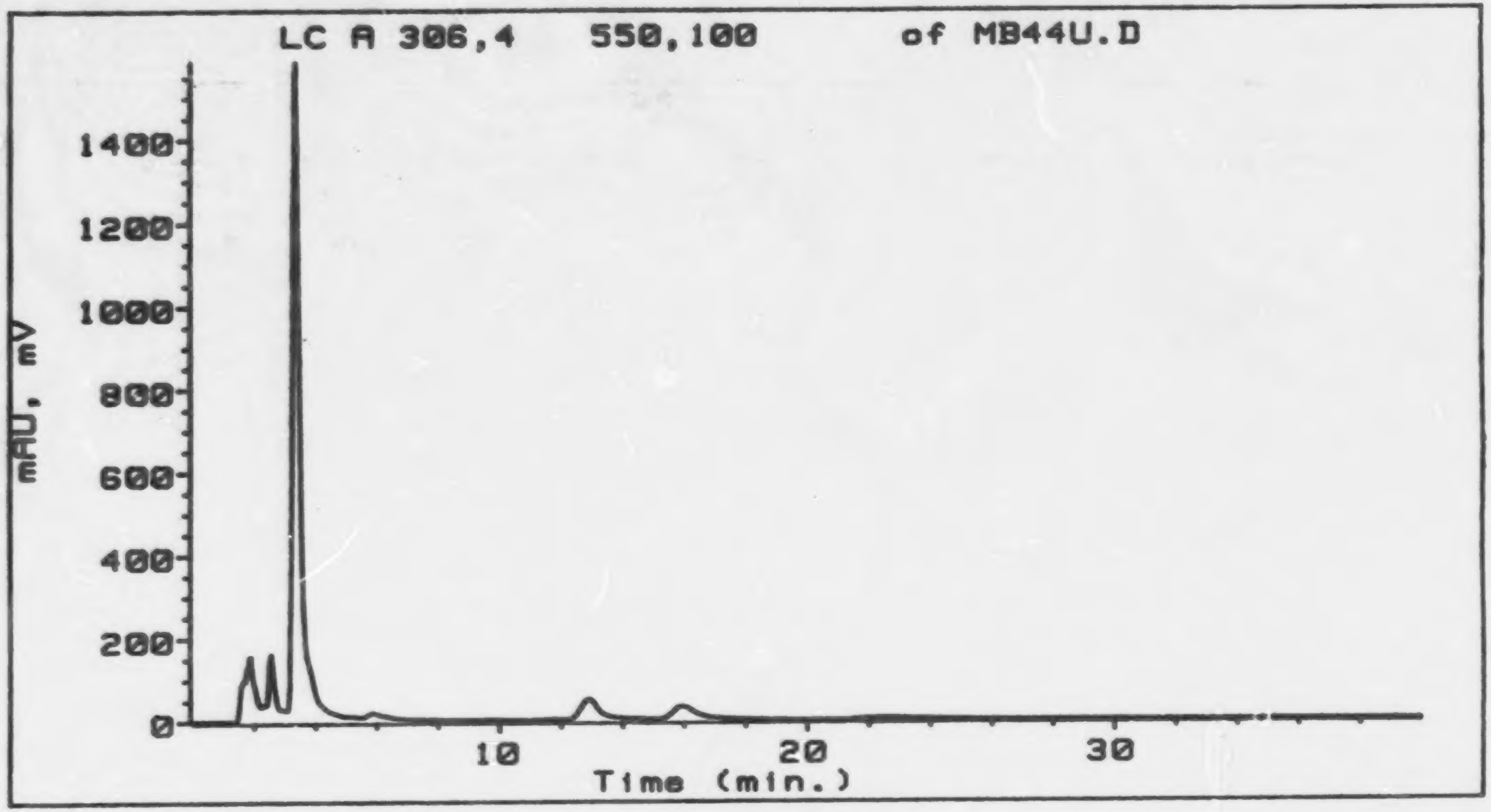

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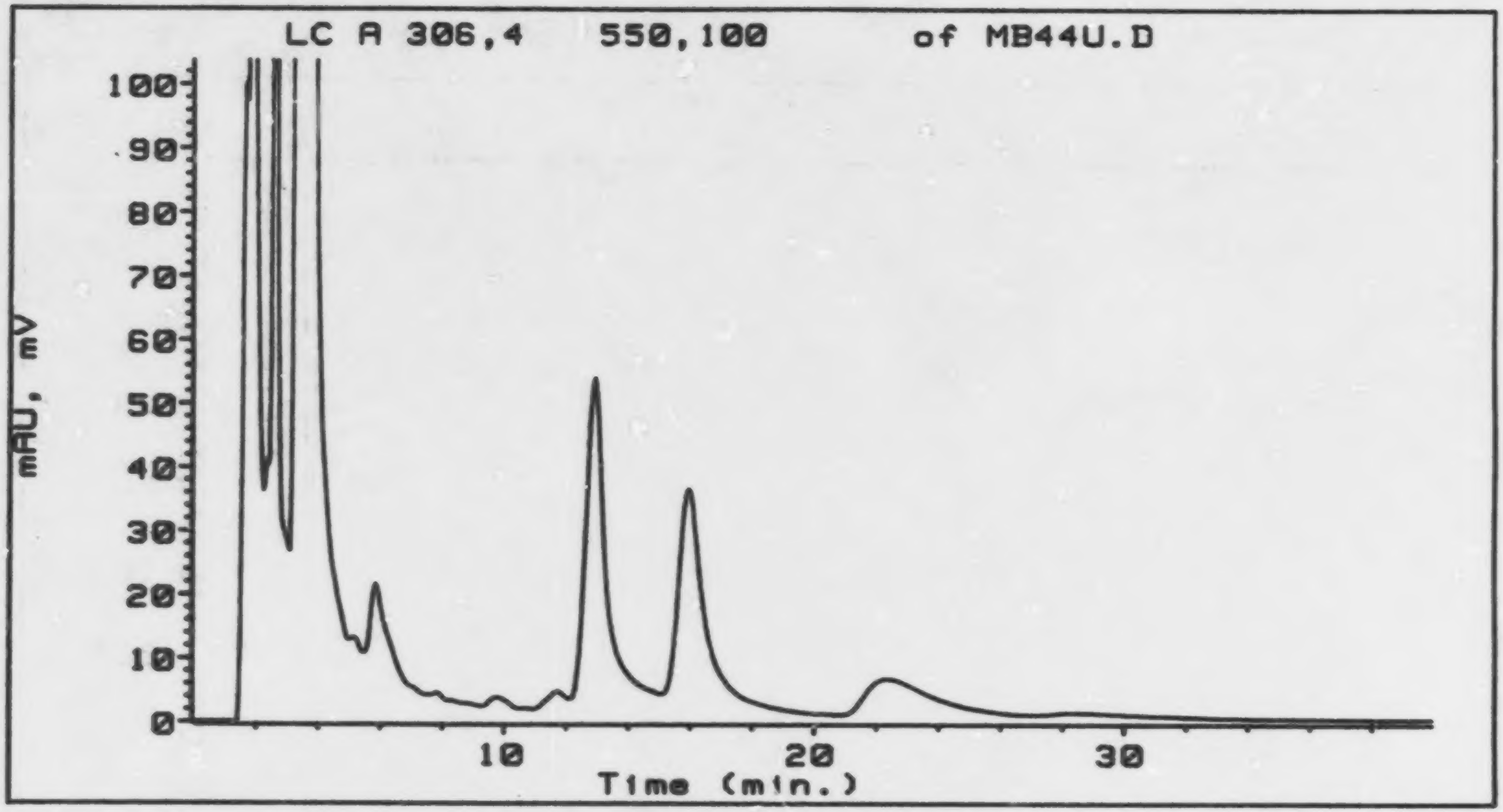

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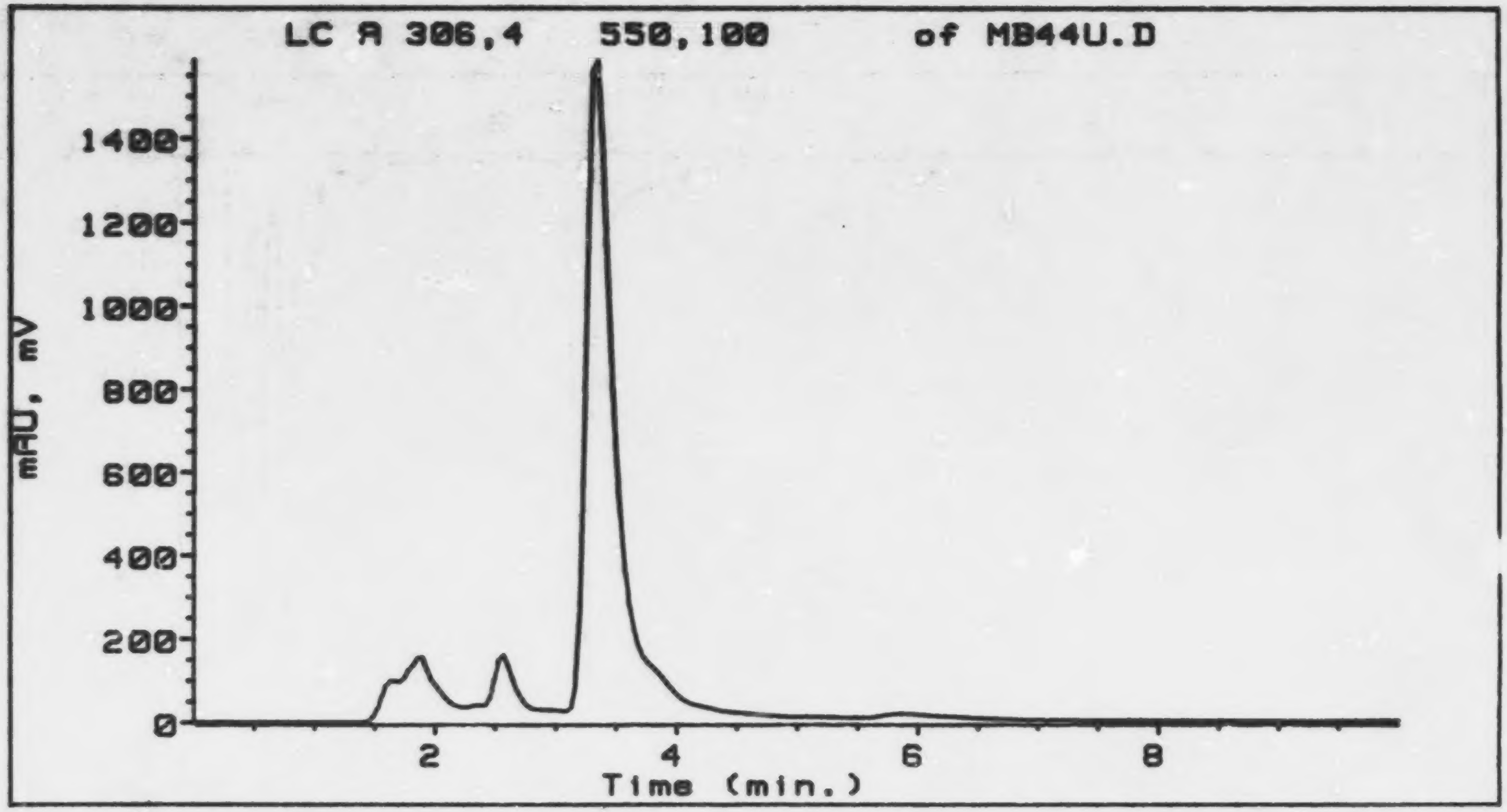

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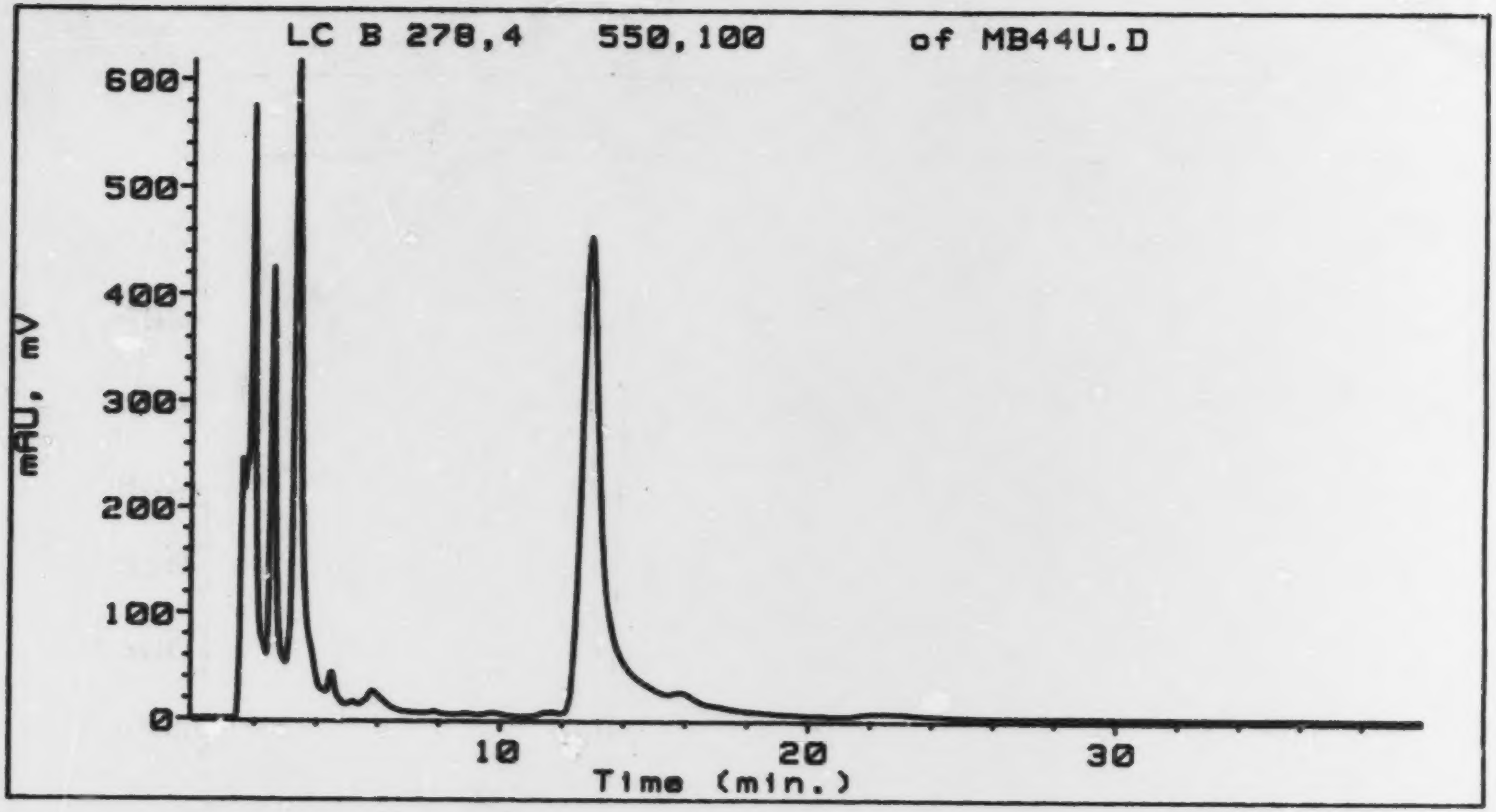

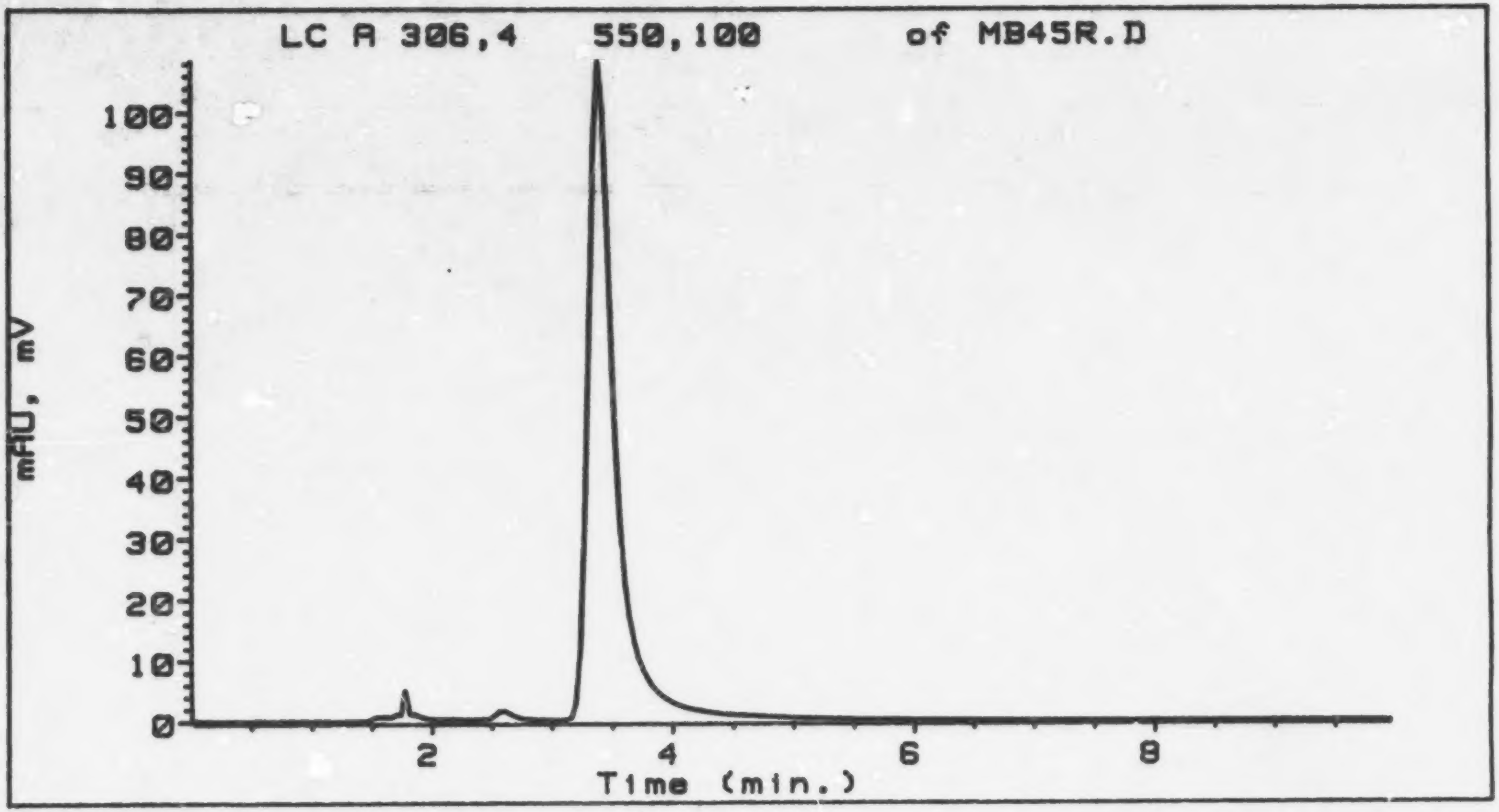

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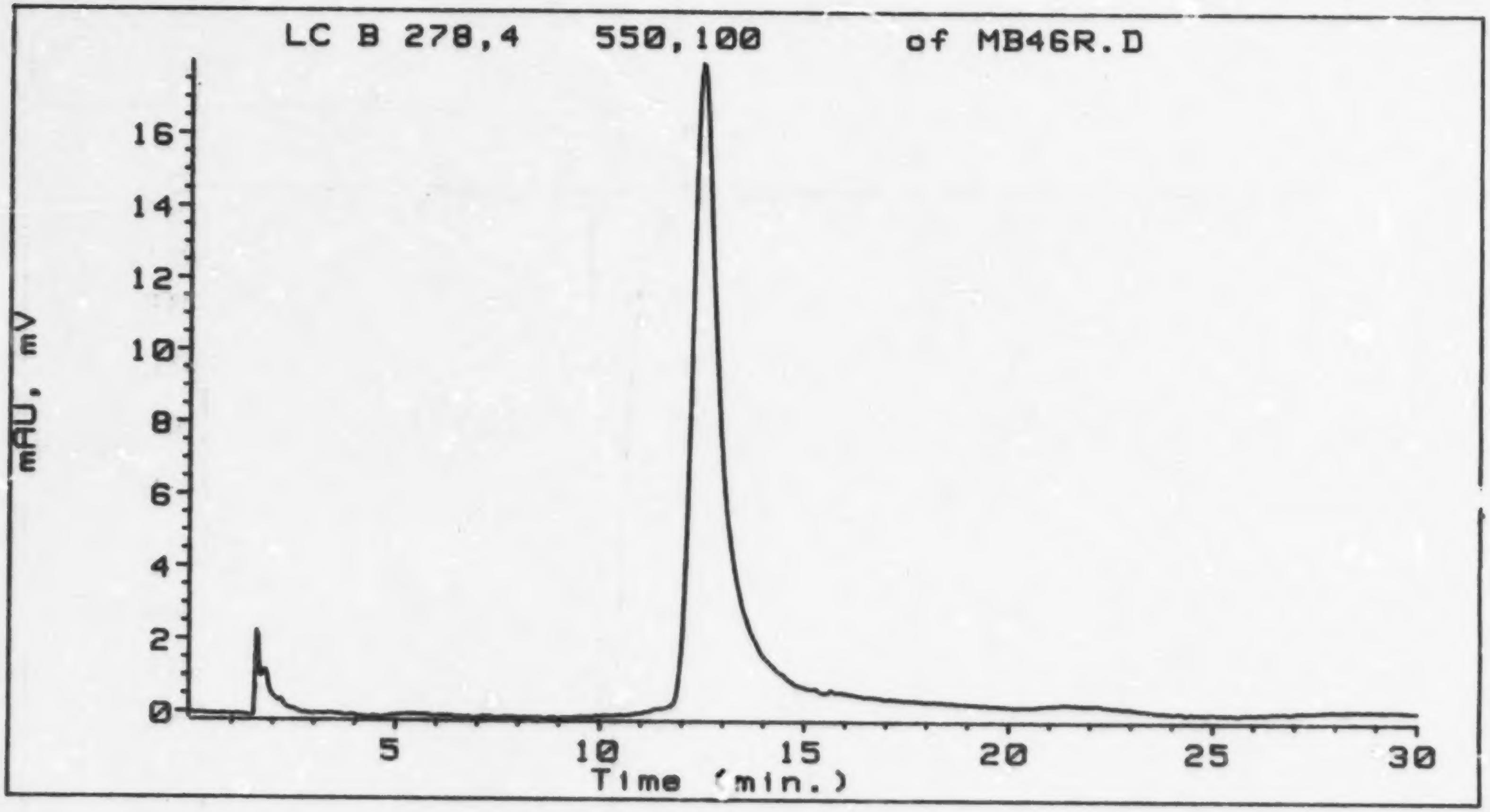

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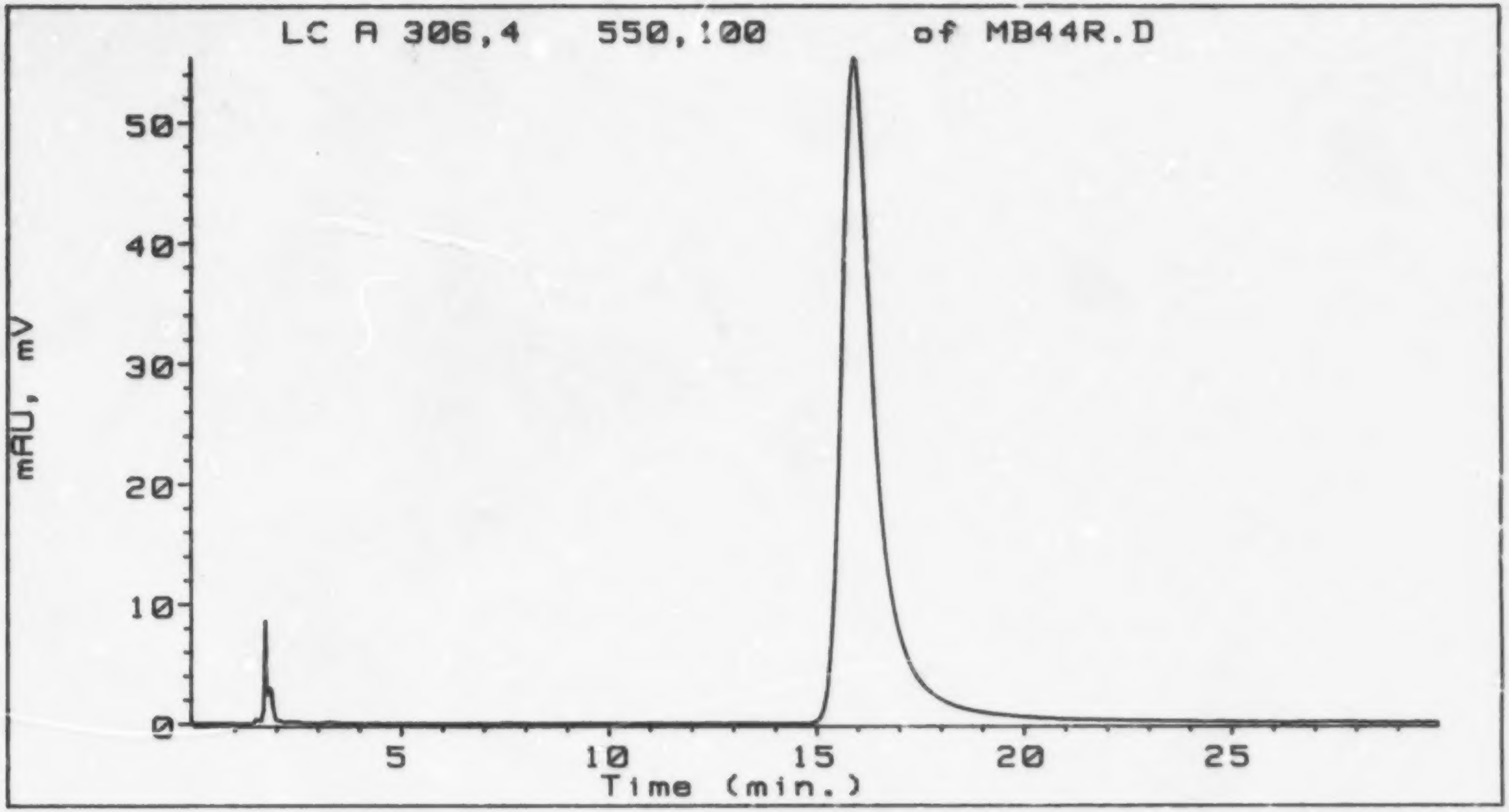

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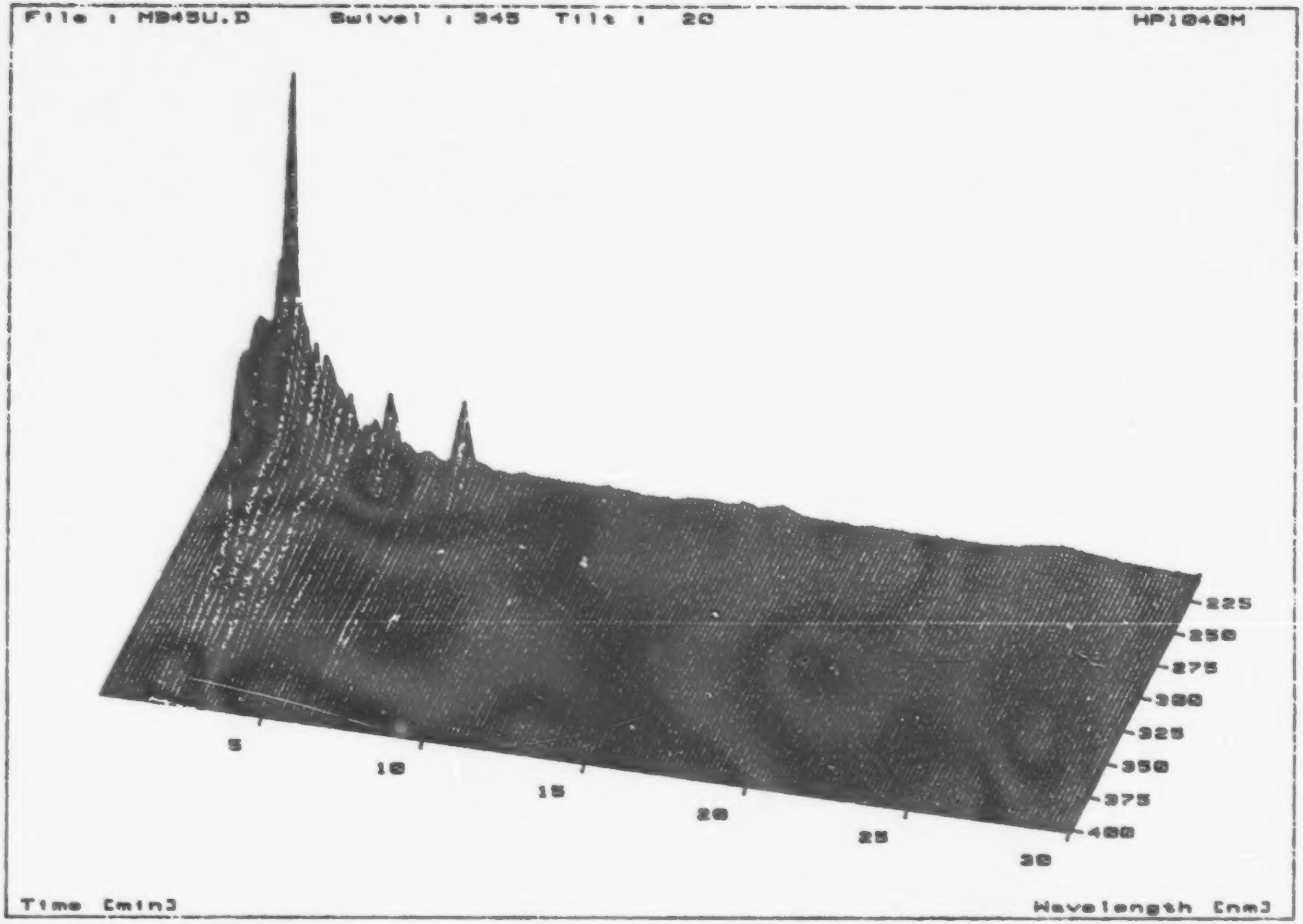

Fig. 22 Three-dimensional chromatogram of patient urine collected prior to dosing obtained using two chromatographic columns in tandem showing the presence of urinary constituents eluting at the retention time of 4'-hydroxymerbarone (3.3 min).

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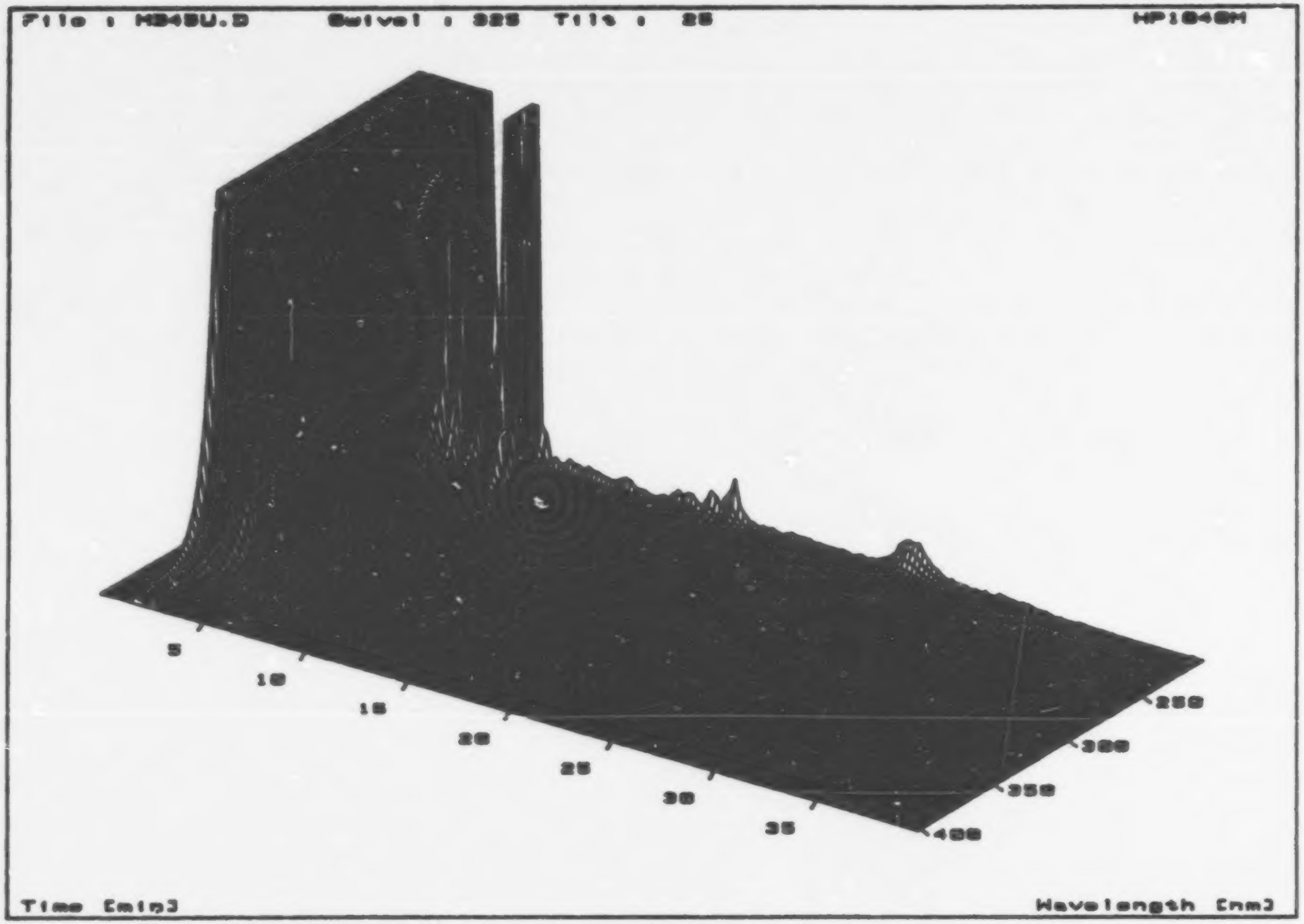

Fig. 23 Three-dimensional chromatogram of patient urine collected prior to dosing obtained using two chromatographic columns in tandem showing that there is a very minor urinary constituent eluting at the retention time of 2-oxo-2-desthiomerbarone (12.9 min) and no interference with the detection of merbarone (15.9 min).

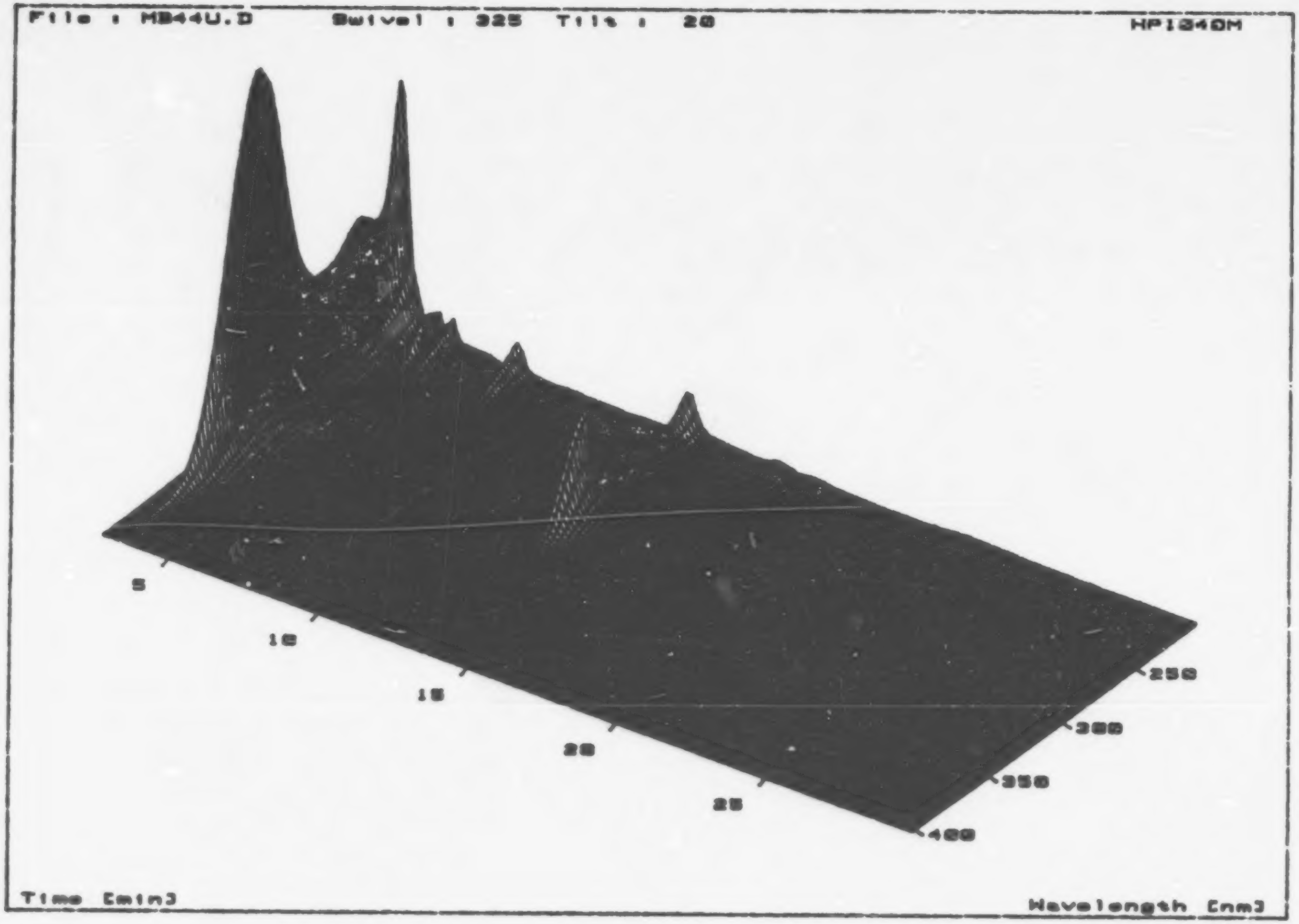

Fig. 24 Three-dimensional chromatogram from 1.8 to 30 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/day x 5 obtained using two chromatographic columns in tandem showing the relative concentrations of the metabolites eluting at 3.3 and 12.9 min to that of merbarone eluting at 15.9 min.

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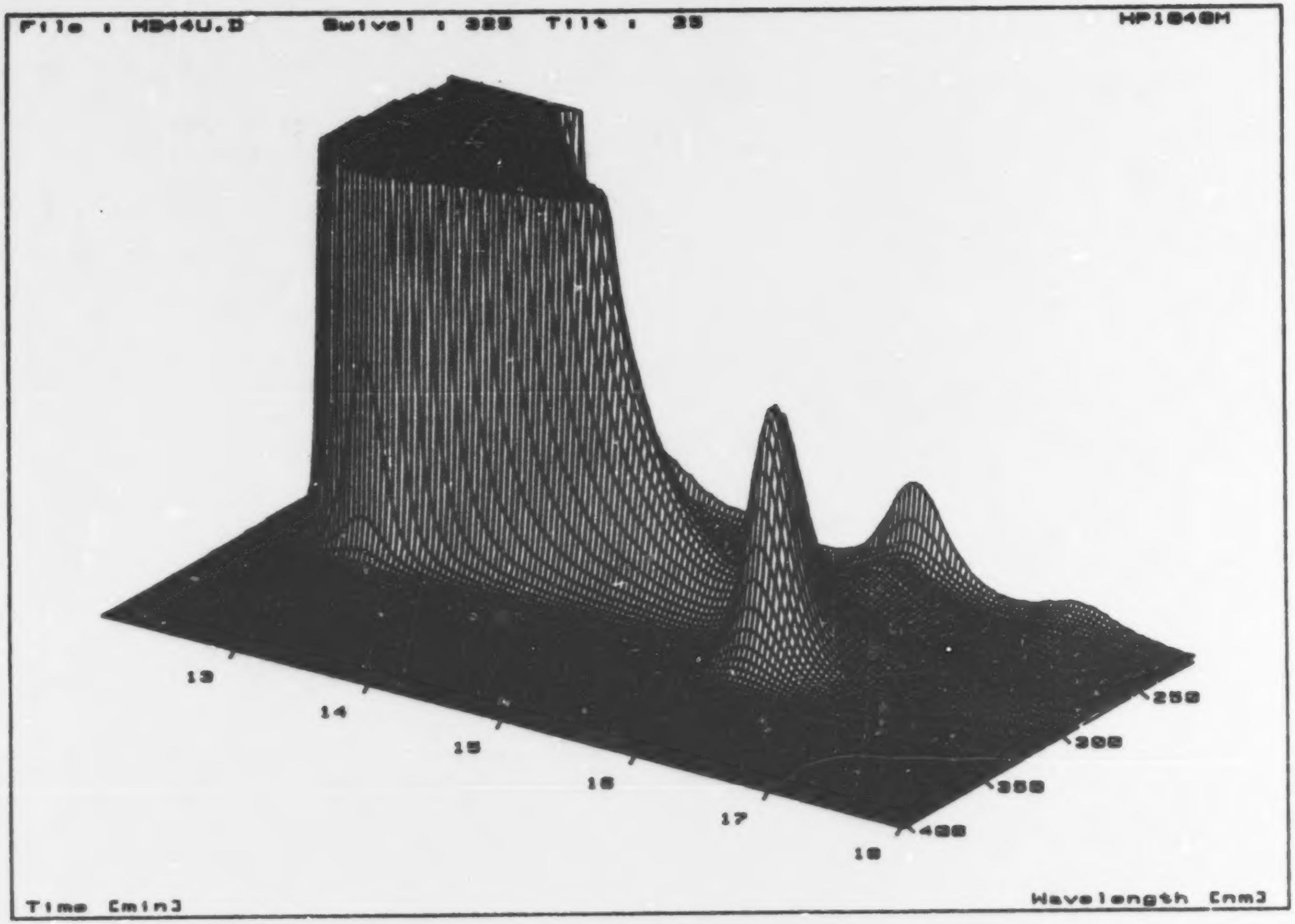

Fig. 25 Three-dimensional chromatogram from 12 to 18 min of patient urine collected on day 5 during the continuous infusion of merbarone 144 mg/m^2/day x 5 obtained using two chromatographic columns in tandem. The metabolite peak (2-oxo-2-desthiomerbarone) eluting at 12.9 min is truncated and both the metabolite and merbarone (15.9 min) peaks exhibit tailing.

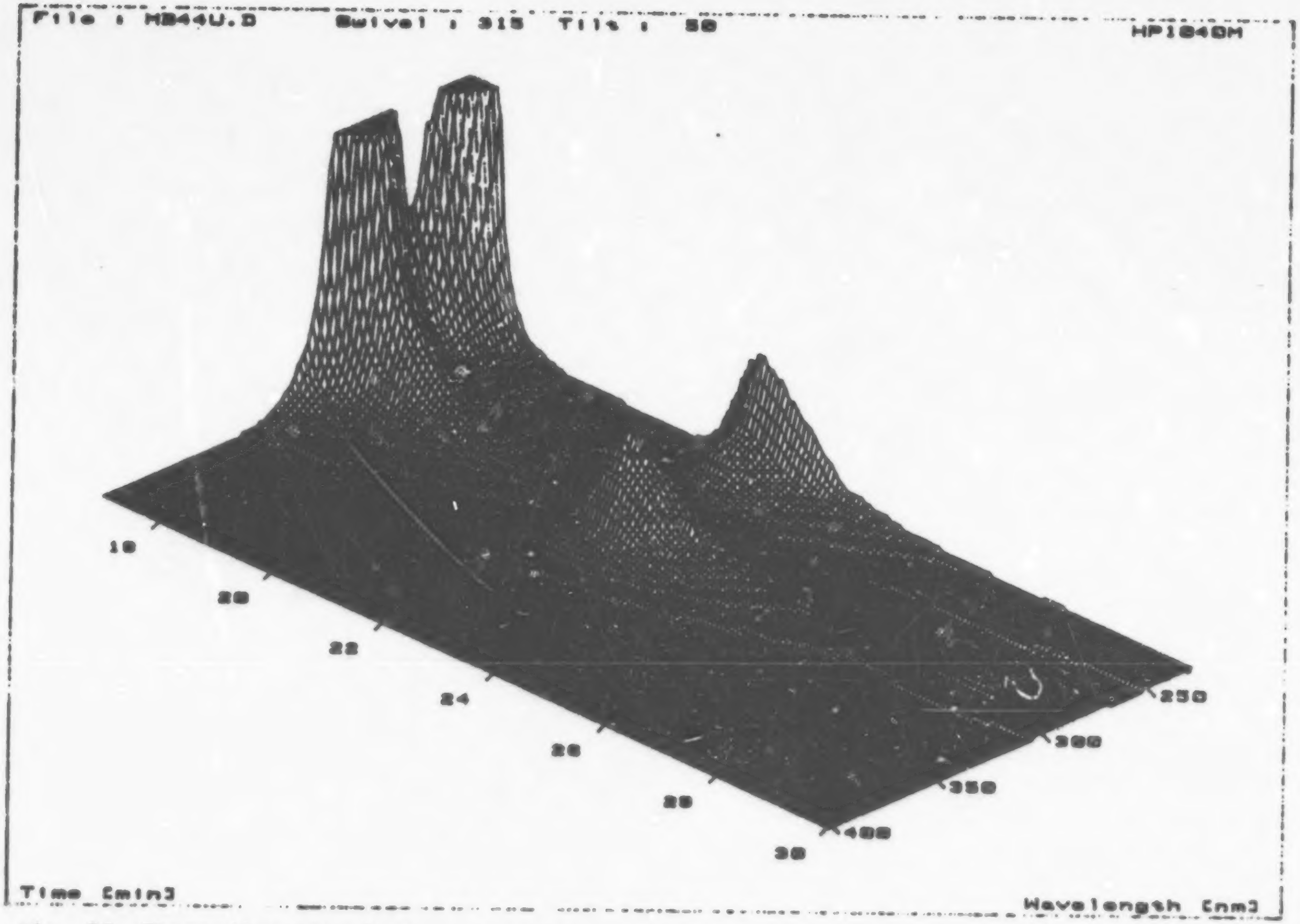

Fig. 26 Three-dimensional chromatogram from 17 to 30 min of patient urine collected on day 5 during the continuous infusion of merbarone obtained using two chromatographic columns in tandem showing the truncated merbarone peak and the minor metabolite eluting at 22.4 min.

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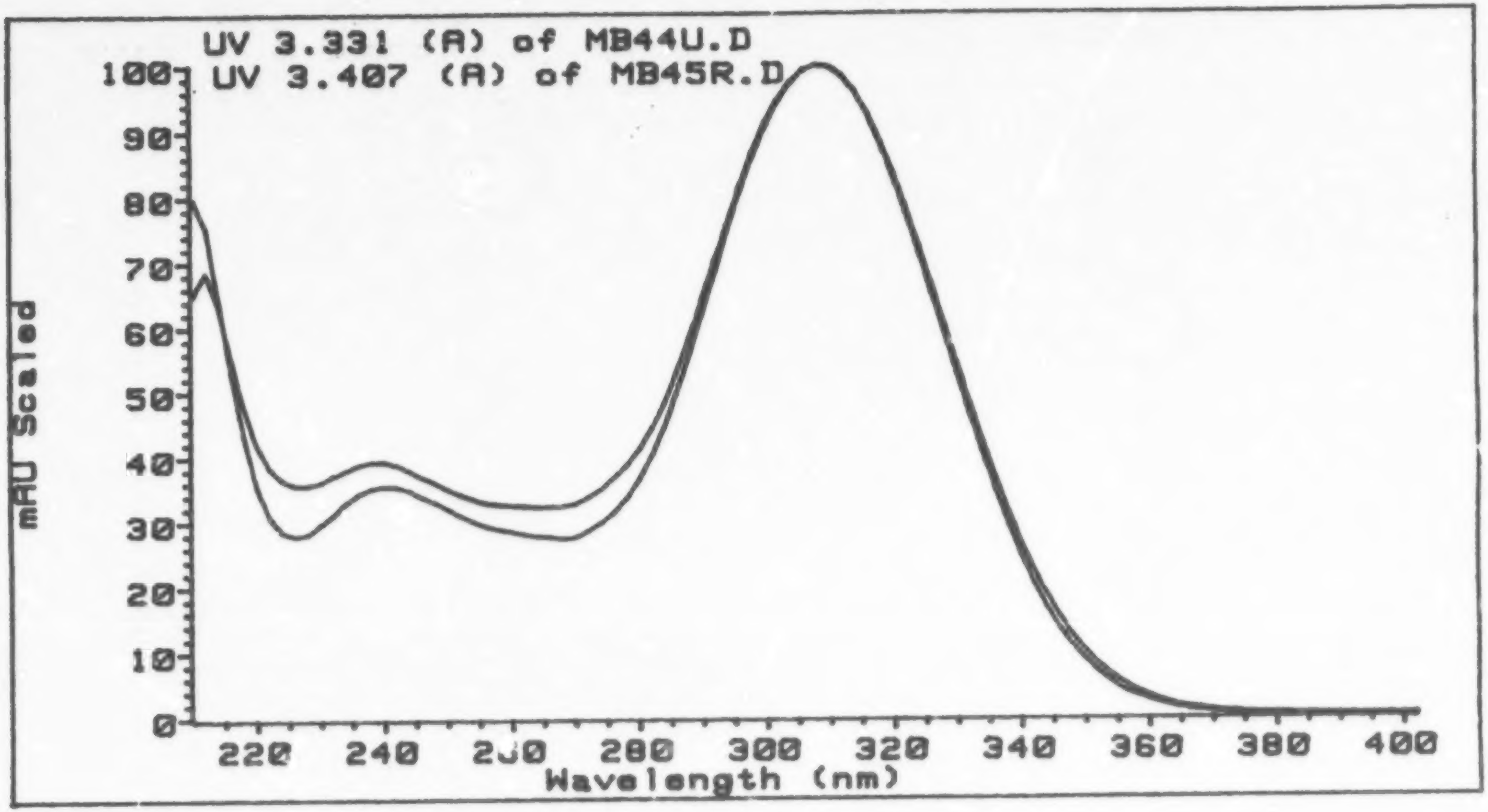

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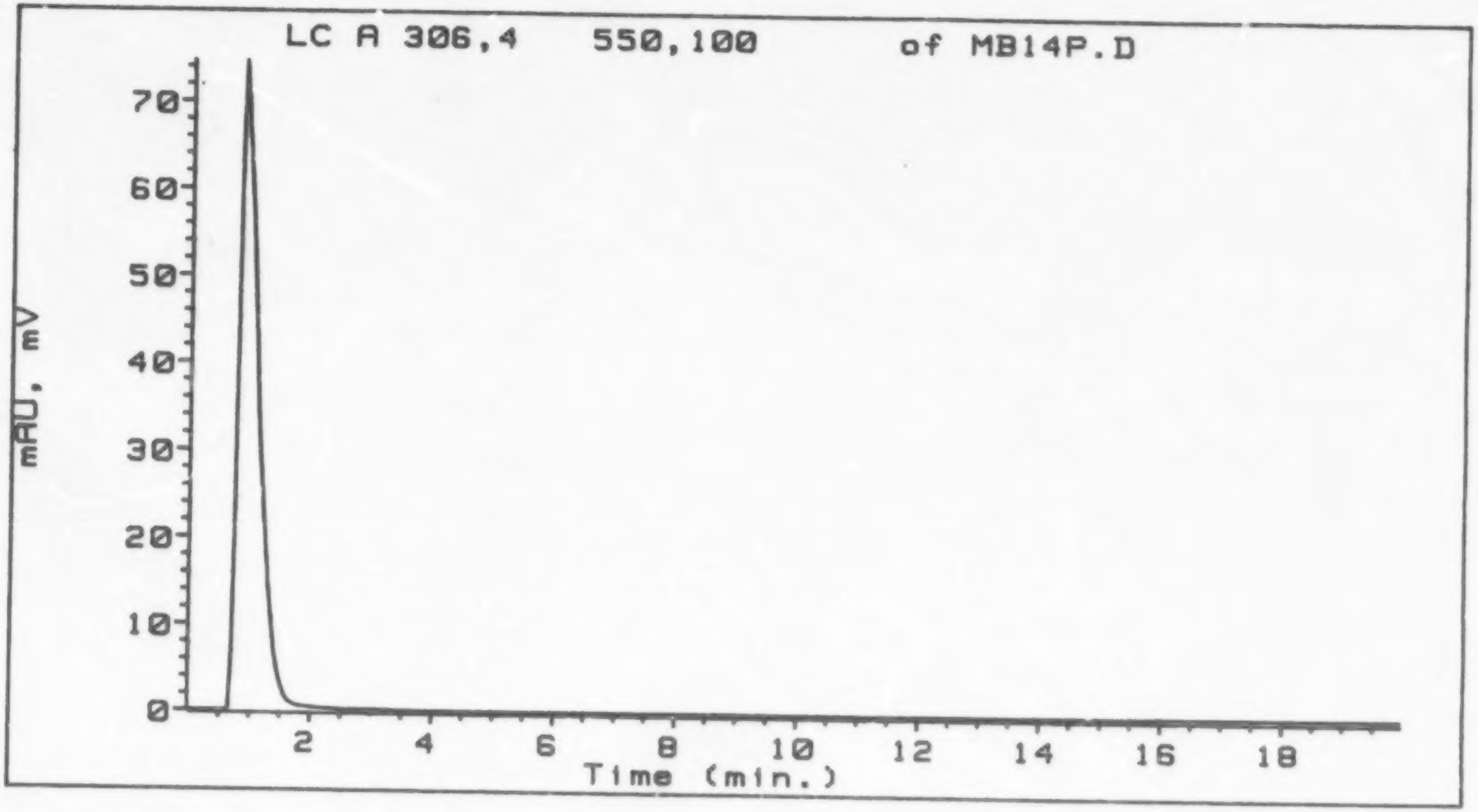

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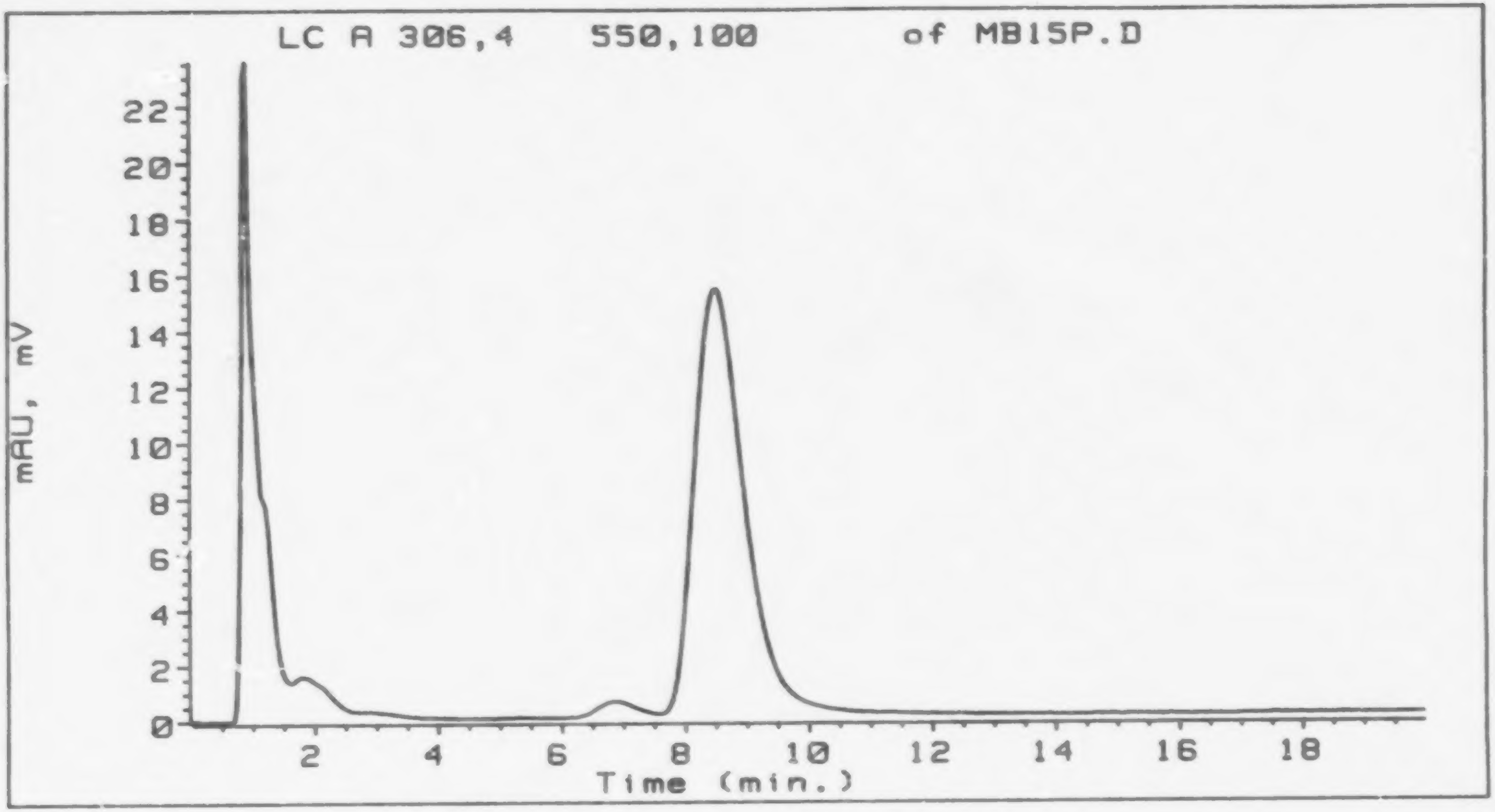

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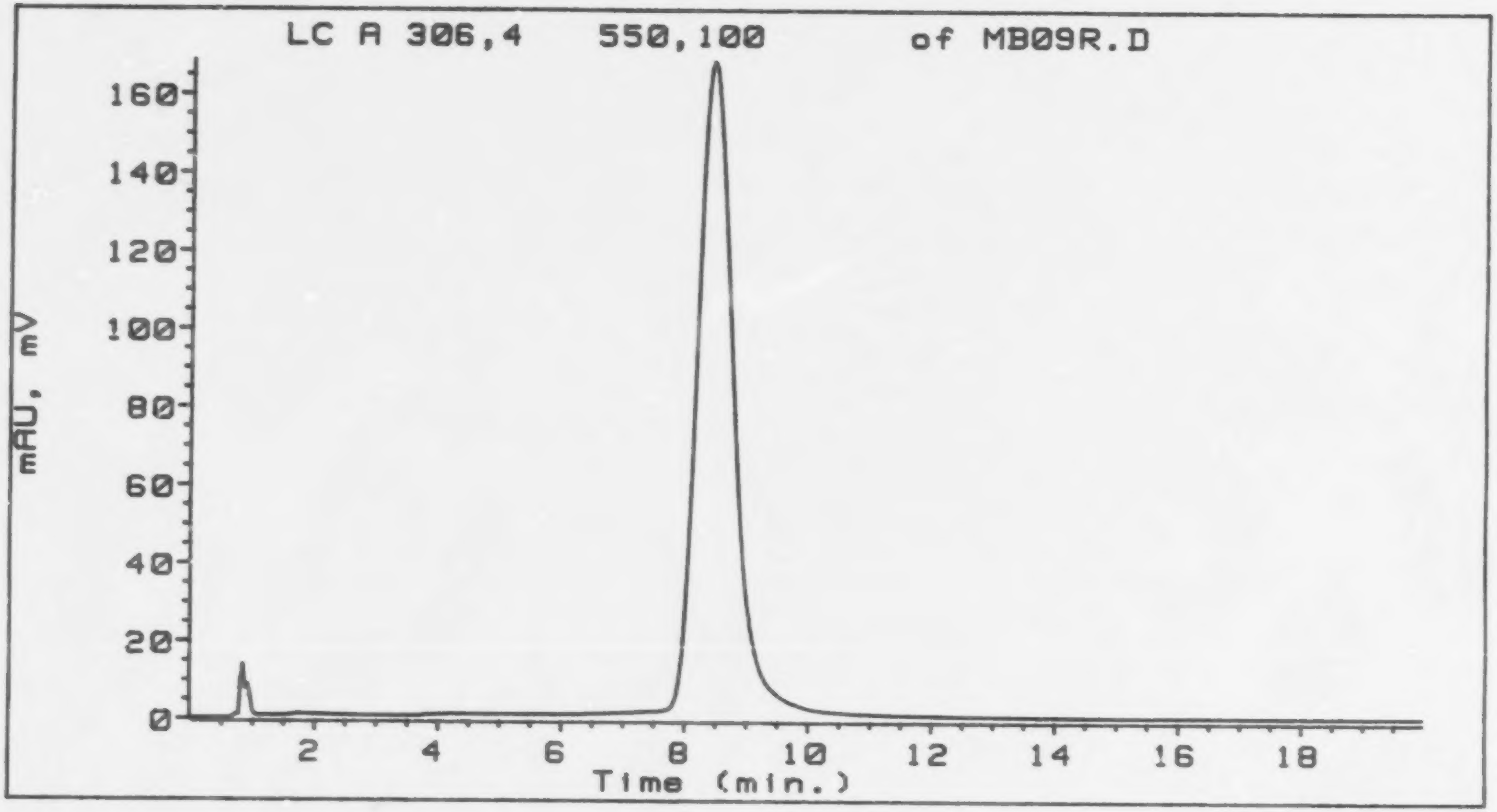

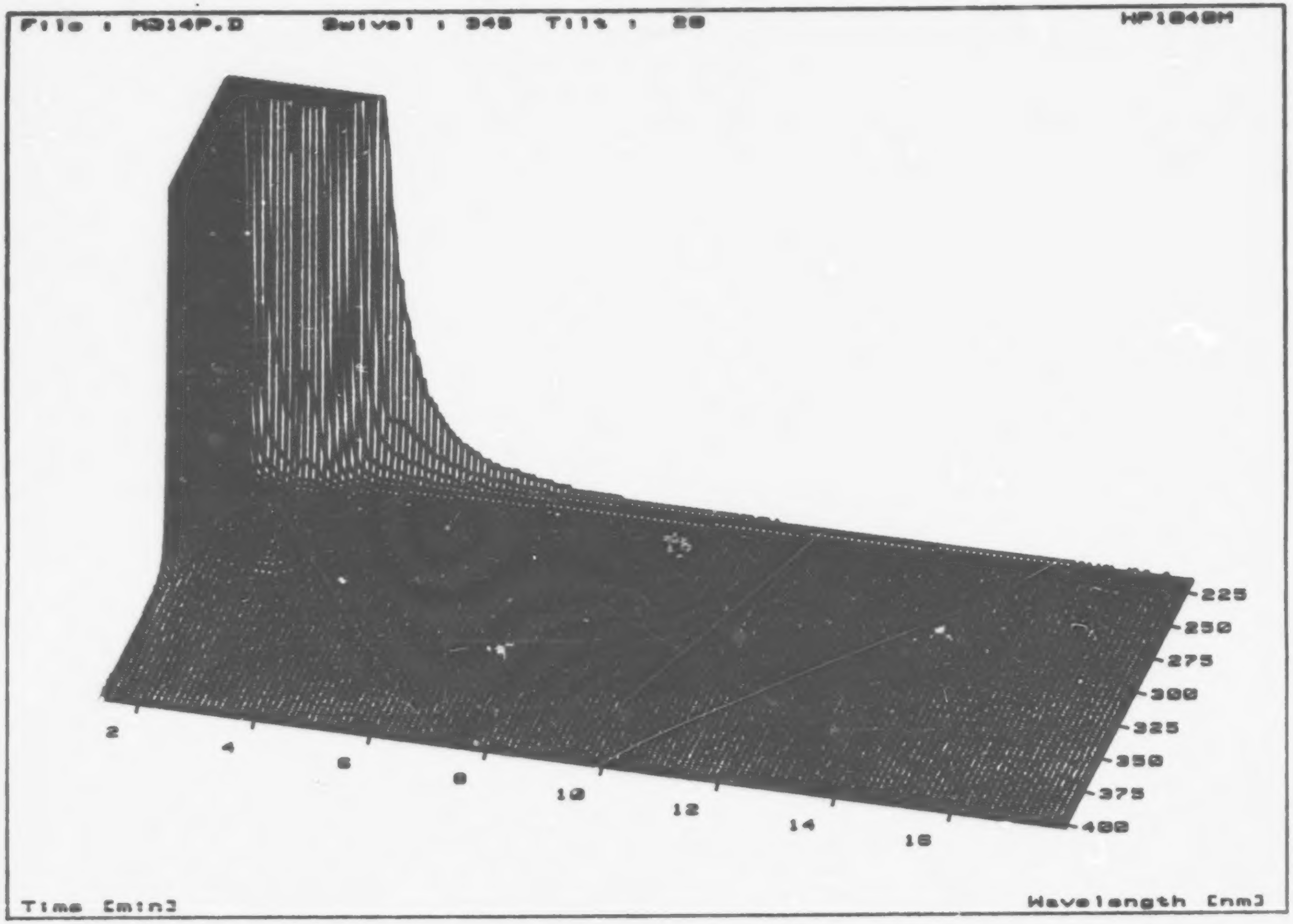

Fig. 31 Three-dimensional chromatogram from 1.4 to 18 min of drug-free patient plasma showing the absence of constituents which would interfere with the detection of merbarone.

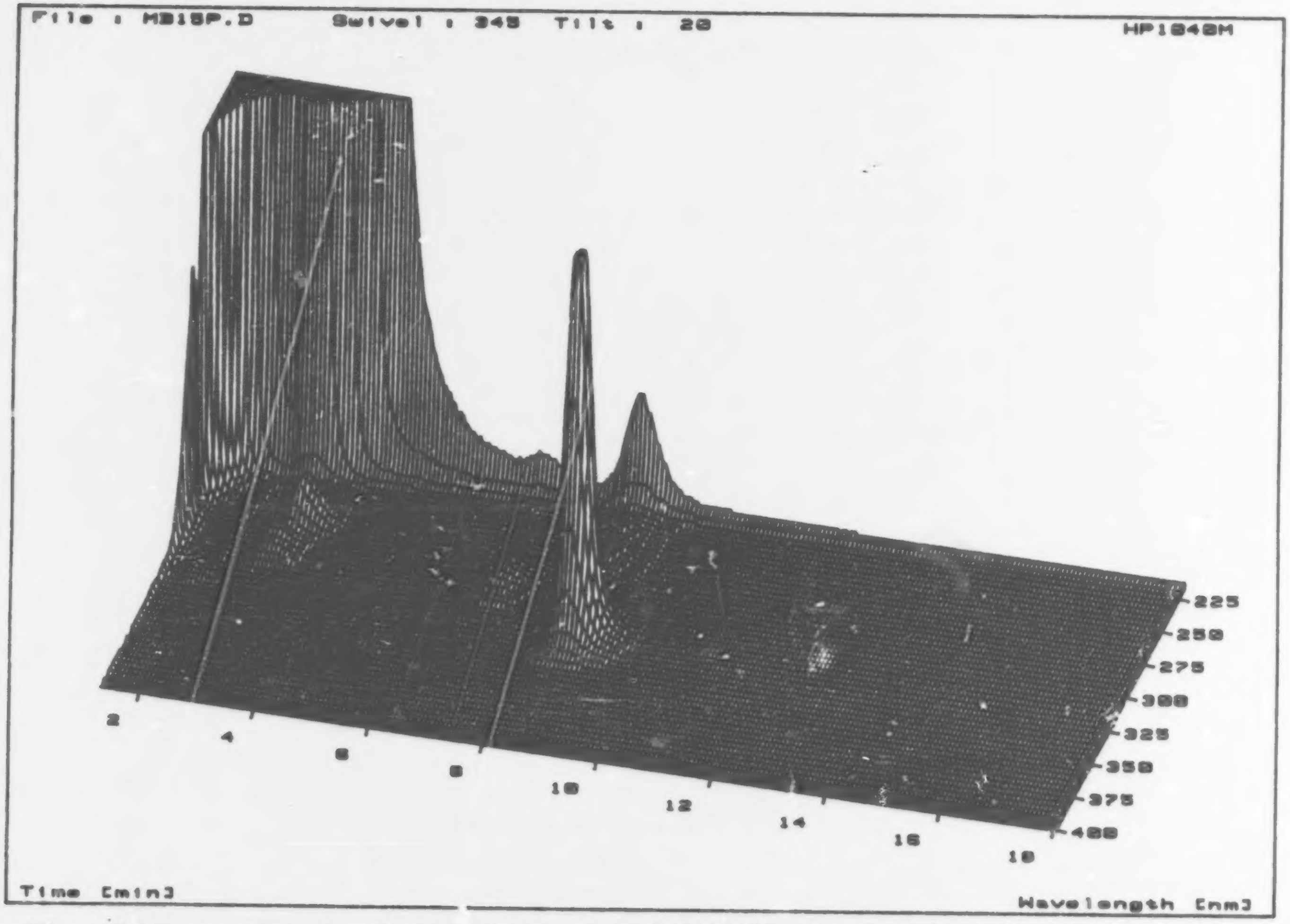

Fig. 32 Three-dimensional chromatogram from 1.4 to 18 min of patient plasma obtained on day 5 during the continuous intravenous infusion of merbarone 144 mg/m^2/day x 5. Chromatographic peaks: (6.9 min) 2-oxo-2-desthiomerbarone, (8.4 min) merbarone.

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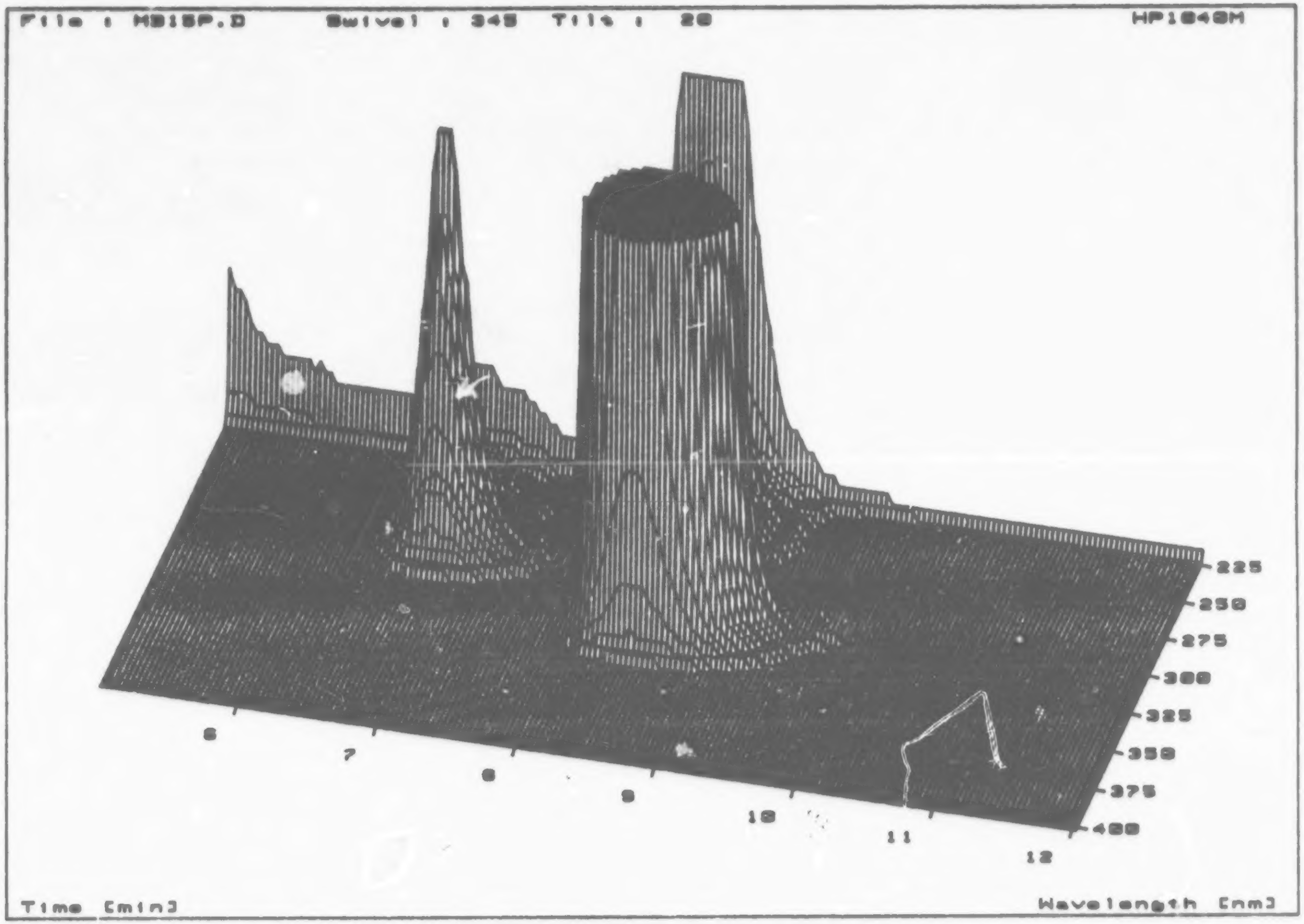

Fig. 33 Three-dimensional chromatogram from 5 to 12 min of patient plasma obtained on day 5 during the continuous intravenous infusion of merbarone 144 mg/m^2/day x 5. The merbarone peak eluting at 8.4 min is truncated and the relatively low concentration of the metabolite, 2-oxo-2-desthiomerbarone (6.9 min) is illustrated.

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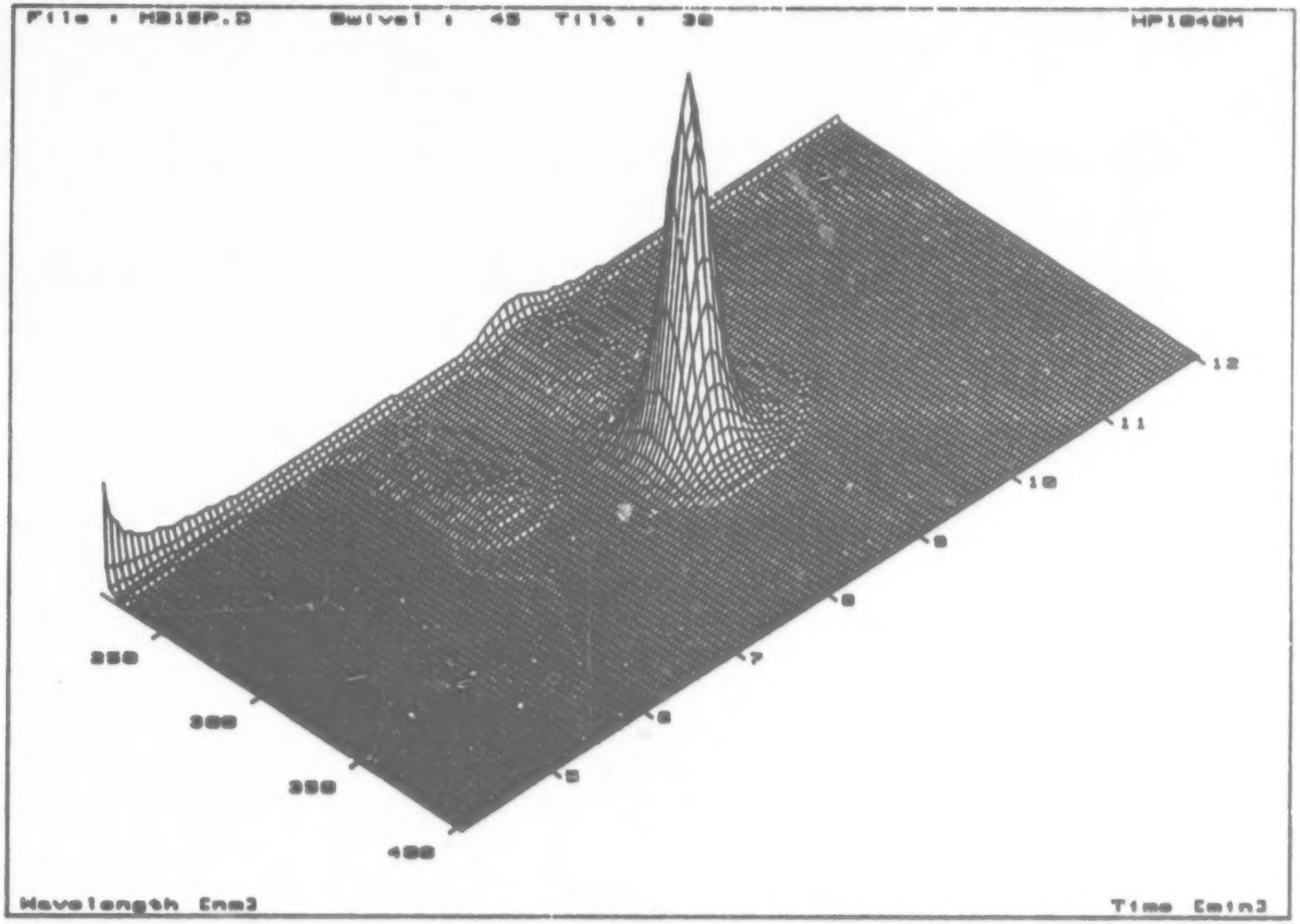

Fig. 34 Three-dimensional chromatogram from 4 to 12 min of day 5 patient (R.F.) plasma illustrating the low level of 2-oxo-2-desthiomerbarone relative to that of merbarone.

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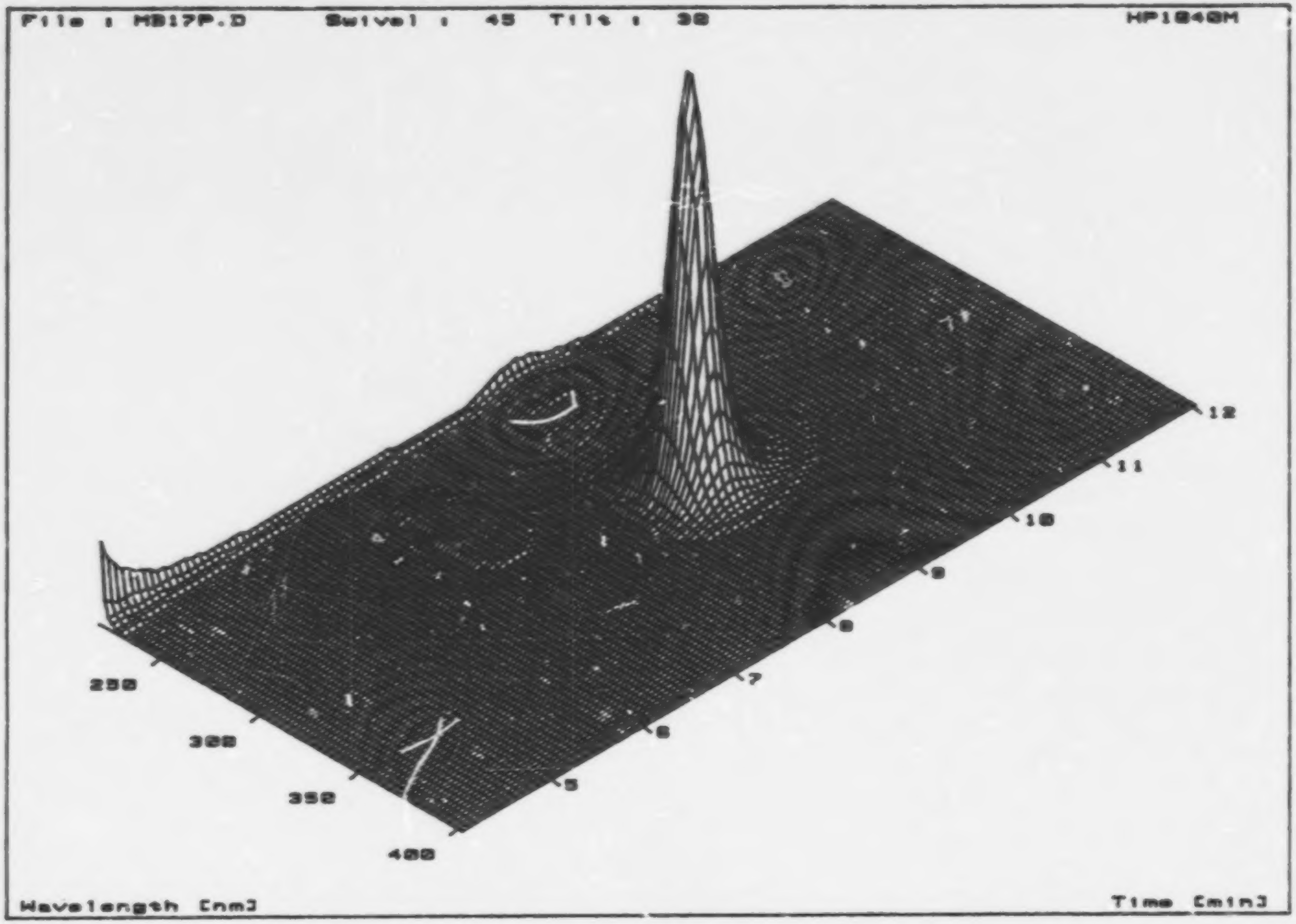

Fig. 35 Three-dimensional chromatogram from 4 to 12 min of day 5 patient (J.B.) plasma illustrating the low level of 2-oxo-2-deschiomerbarone relative to that of merbarone.

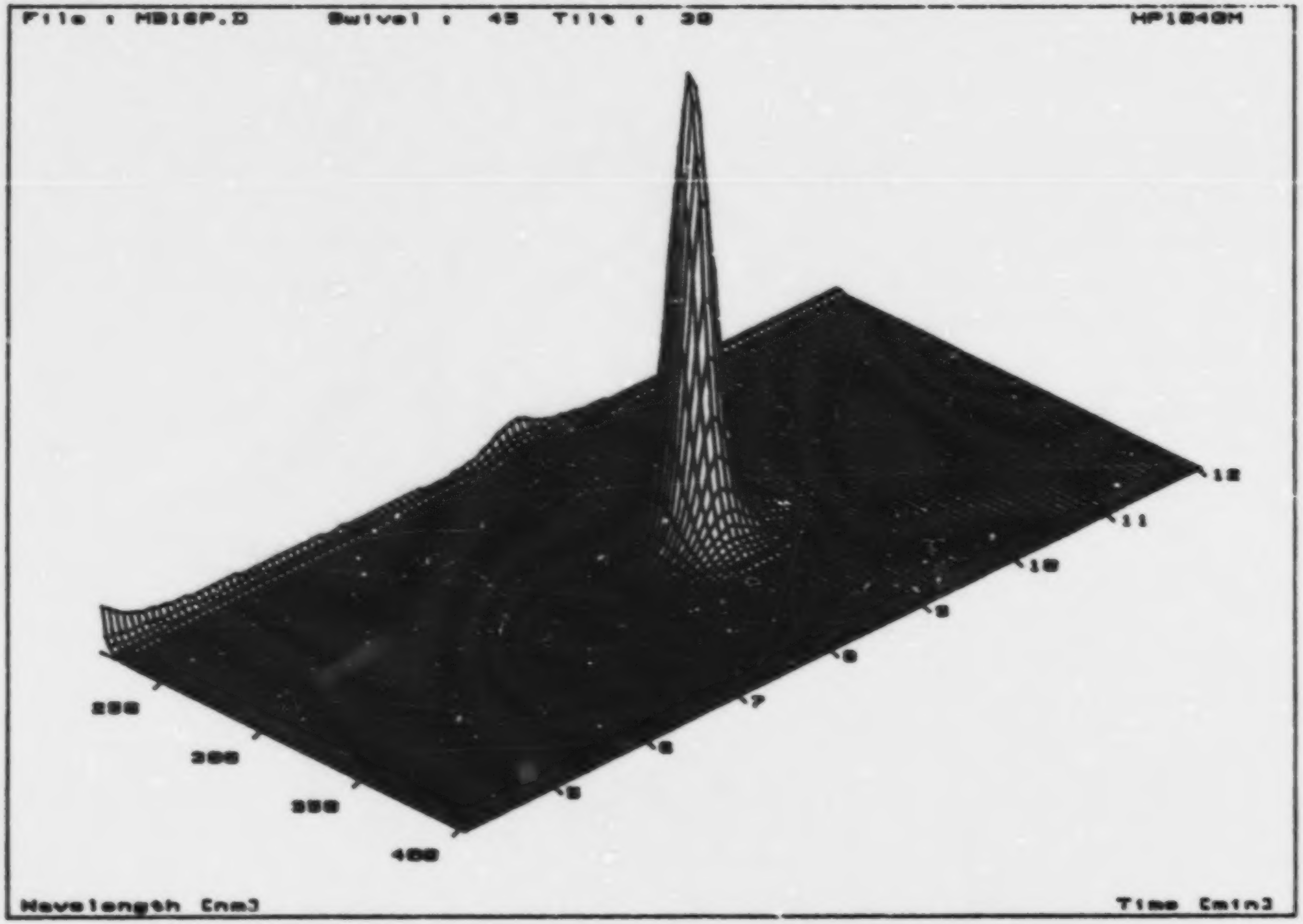

Fig. 36 Three-dimensional chromatogram from 4 to 12 min of day 5 patient (H.S.) plasma illustrating the low level of 2-oxo-2-desthiomerbarone relative to that of merbarone.

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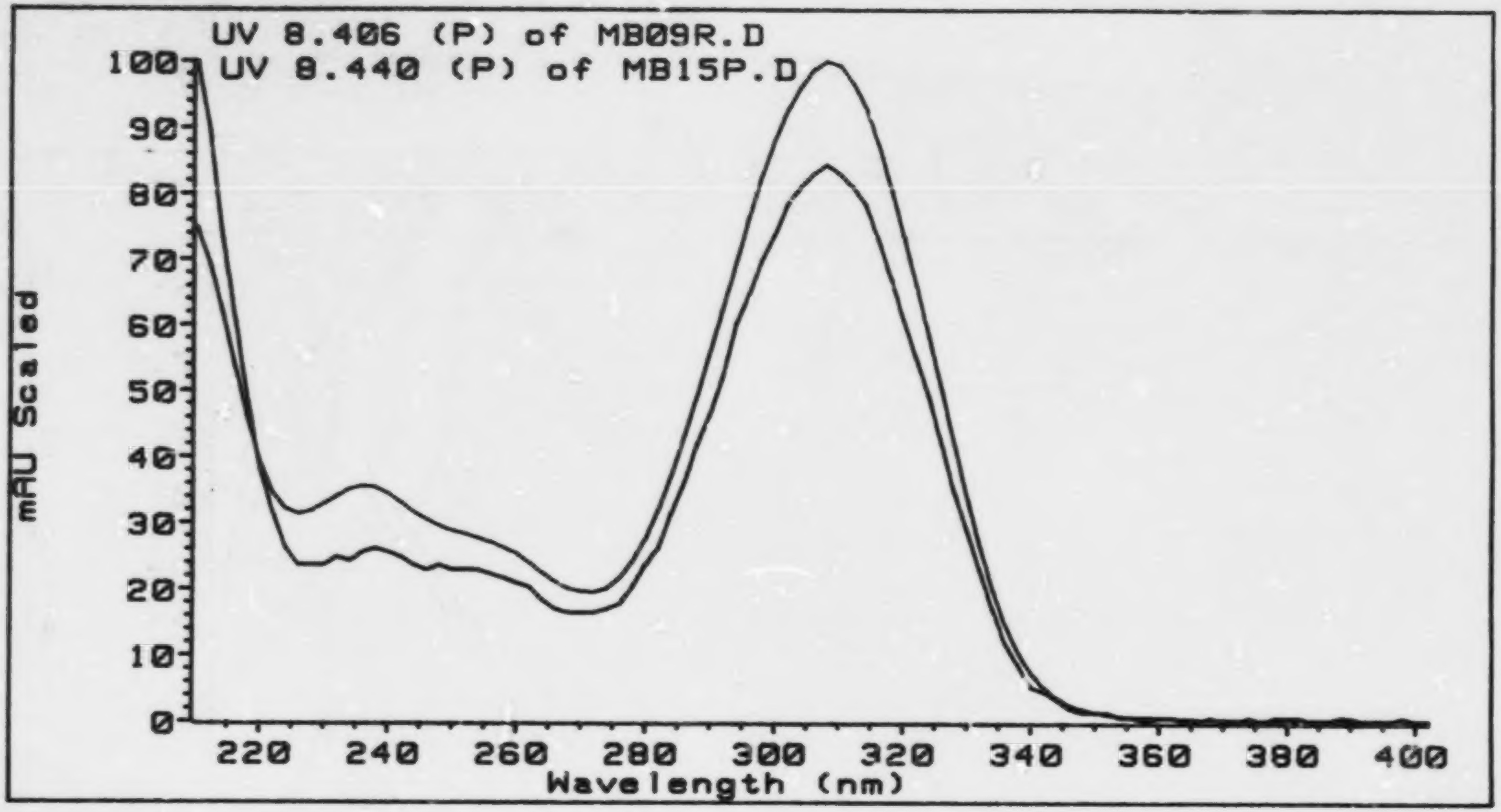

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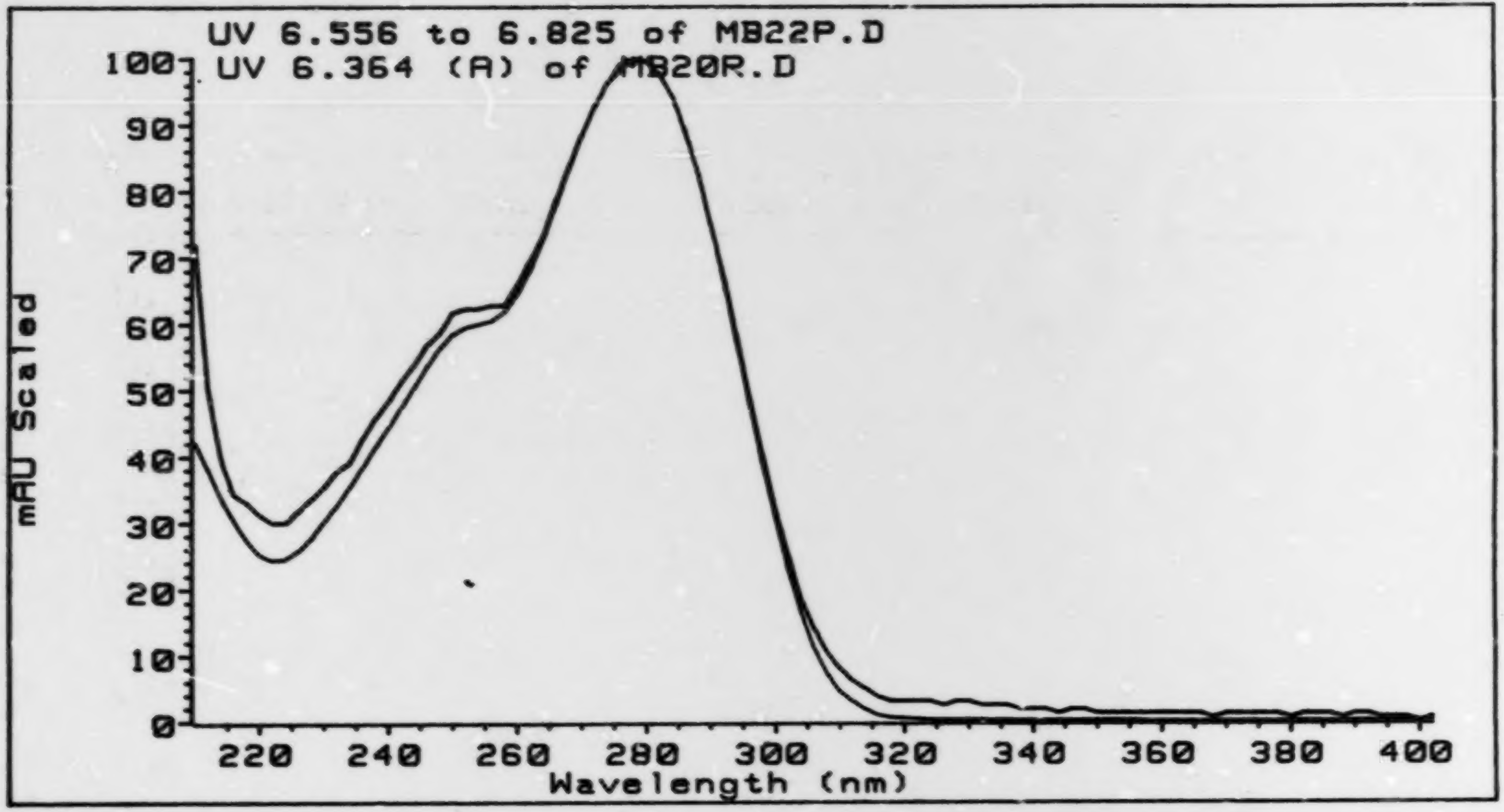

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PHARMACOKINETIC AND METABOLIC SUMMARY

1. THE TOTAL BODY PLASMA CLEARANCE, CL_T, OF MERBARONE DURING THE 5 DAY CONTINUOUS INFUSION OF 96 MG/M^2/DAY AND 144 MG/M^2/DAY RANGED FROM 8.0 TO 14.4 ML/MIN/M^2 FOR 6 PATIENTS.

2. FOLLOWING THE END OF THE INFUSION, THE DECLINE OF THE PLASMA LEVELS, MONITORED TO 60 HR AFTER THE 144 MG/M^2/DAY X 5 DOSE, APPEARED TO BE BIPHASIC WITH $T_{1/2\alpha}$ RANGING FROM 0.8 TO 3.7 HR AND $T_{1/2\beta}$ RANGING FROM 9.2 TO 14.6 HR.

3. MERBARONE IS NOT HIGHLY BOUND TO THE TISSUES WITH VD_{SS} RANGING FROM 5.6 TO 8.7 L/M^2 (15 TO 24 % OF BODY WEIGHT).

4. ELIMINATION IS PRIMARILY NON-RENAL. THE RENAL EXCRETION WAS APPROXIMATELY 2% OF THE DOSE AND RANGED FROM 0.3 TO 4.3% IN THE 6 PATIENTS. THE VERY LOW RENAL EXCRETION IS PROBABLY DUE TO THE HIGH DEGREE OF PLASMA PROTEIN BINDING.

5. THE TOTAL BODY CLEARANCE OF MERBARONE IN MAN IS CONSIDERABLY SMALLER THAN REPORTED FOR THE DOG. THEREFORE, THE STEADY STATE PLASMA LEVELS OBSERVED IN MAN WERE HIGHER THAN EXPECTED.

6. CHROMATOGRAMS OF PATIENT URINE SHOW THE PRESENCE OF TWO MAJOR METABOLITES. THE CONCENTRATIONS OF THESE METABOLITES IN URINE ARE SUBSTANTIALLY GREATER THAN THAT OF THE PARENT DRUG.

7. THE METABOLITES EXHIBIT LC RETENTION TIMES AND UV SPECTRA SIMILAR TO 4'-HYDROXYMERBARONE AND 2-OXO-2-DESTHIOMERBARONE.

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Phase I and Clinical Pharmacologic Study of Merbarone

Raymond P. Warrell, Jr. M.D.

7/7/86

Merbarone was administered to 10 patients with various types of cancer. The drug was administered as a continuous i.v. infusion daily for 5 days. The initial dose (100 $mg/m^2/d \times 5$) approximated one-third of the lowest non-toxic dose in dogs. A relatively small dose increment between levels was established after measurements revealed unexpectedly high plasma drug concentrations (4-6 μg/ml) at the lowest dose.

One patient has been treated at the highest dose level, 250 $mg/m^2/d$. Mild nausea without vomiting occurred in this patient accompanied by a pruritic skin rash over the upper torso. Both reactions are only possibly drug-related. At doses $\leq$ 200 $mg/m^2/d \times 5$, the most prominent reaction was phlebitis at the infusion site. Mild nausea and fatigue, possibly drug-related, have also been observed. One patient with gastric cancer and Crohn's disease developed moderate diarrhea after the first infusion at 200 $mg/m^2/d \times 5$. Out-patient administration of i.v. fluids was required. No other significant GI or CNS reactions have been noted. No major responses have been seen to date.

Pharmacologic studies (see notes from Dr. C. Young below) have shown steady-state plasma drug concentrations up to 16 μg/ml. It has been noted that lethal CNS toxicity occurred in one dog with steady-state plasma levels approximating 18 μg/ml. Therefore, further escalation will continue cautiously at increments of 50 $mg/m^2/d$. Patients who receive the drug through peripheral venous catheters will also receive additional i.v. fluids in an effort to ameliorate the phlebitis at infusion sites.

TABLE 1

Trial Design

- Continuous Infusion
- Daily x 5 Days
- Initial Dose = 100 $mg/m^2/day$
- Repeated ~ Q 21 Days
- No escalation in the same patient

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TABLE 2

Patient Characteristics

Histologic Diagnoses:	N=10
Lung (NSC)	3
Kidney	1
Bladder	1
Breast/Gastric	1
Penis	1
Sarcoma	1
Lymphoma	1
Thymoma	1
Performance Status	70 (50-90)
Age	45 (33-74)
Prior Chemotherapy	10/10
Prior Radiotherapy	6/10

TABLE 3

Dose Escalation

Dose (mg/m^2/d x 5)	Patients Entered	Patients Evaluable	Pending Evaluation	Inevaluable
100	3	3	–	–
150	3	3	–	–
200	3	2	1	–
250	1	–	1	–

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TABLE 4

Toxicity

Dose (mg/m^2/d x 5)	Phlebitis	Vomiting	Diarrhea	Weakness	CNS
100	0/3	1/3	–	2/3	–
150	3/3	0/3	–	–	–
200	-/-	1/3	1/3	1/3	–
250	-/-	1/1	–	–	–

TABLE 5

Hematologic Toxicity

Dose (mg/m^2/d x 5)	WBC x 1000 Initial	WBC x 1000 Nadir	Platelets x 1000 Initial	Platelets x 1000 Nadir
100	10.6 (8.7-14.8)	5.4 (5.1-10.6)	414(280-590)	341(208-440)
150	11.8 (7.4-23.1)	10.0 (7.5-24.5)	304(294-521)	379(287-404)
200	6.9 (5.7-10.4)	5.6 (4.2-9.9)	430(282-556)	275(230-417)
250		≠	446	≠

* Median (range), 1st course only
≠ Too early

TABLE 6

Therapeutic Effects

Disease	Dose	Response	Duration (mos)
Thymoma	100 x 5	Stable	3½+
Lung (NSC)	100 x 5	Stable	3+

≠ Evaluable for Response = 7

TABLE 7

Conclusions

1) Well-tolerated by 5 day continuous infusion
2) No major toxicity to dose of 250 mg/m^2/d x 5
3) No evidence of cumulative toxicity
4) Phlebitis at injection site with doses $\geq$ 150 mg/m^2/d

CLINICAL PHARMACOKINETIC STUDIES OF MERBARONE AT MEMORIAL SLOAN-KETTERING CANCER CENTER

July 9, 1986

Charles W. Young, M.D.
T. Declan Walsh, M.D.

INTRODUCTION

Merbarone was given by a continuous intravenous infusion over 5 days, a schedule chosen because of the increased antitumor effect of the drug on frequent administration, and the acute central nervous system toxic effects of the drug in animals given bolus injections. Preclinical toxicology studies of the continuous infusion schedule showed necrosis of the intestinal mucosa, lymph nodes, spleen, bone marrow and thymus in lethally intoxicated dogs. In rats the drug also produced renal injury.

Detailed pharmacokinetic studies of merbarone in dogs by continuous infusion were carried out at dosage rates of 0.6 to 4.6mg/kg/hr = 12 to 92mg/m2/hour = 288 to 2208mg/m2/day. At least two (polar) metabolites of merbarone have been identified including: 1) a glucuronide of merbarone, and 2) a metabolite with the chromatographic characteristics of 4'-OH merbarone (Malspeis et al, Proc. AACR 27:402, 1986).

In animals dying within 5 hours (500mg/m2/hr) or 30 hours (160mg/m2/hr) plasma merbarone concentrations of >150μg/ml and 90μg/ml were observed. One animal who received 2208mg/m2/day x 5 days, survived the infusion with CP_{ss} = 18.4μg/ml, but expired of toxicity on Day 8.

The elimination phase half-life of merbarone in three studies in dogs ranged from 2.3 to 4 hours. The plasma clearance ranged from 0.102 to 0.447/l/h/kg or 34 to 149/ml/min/m2. AUC exposure ranged from 161 to 2,159mg/l·hr; AUC exposures of 1,888 and 2,159 were associated with toxicity.

CLINICAL STUDIES

Detailed pharmacokinetic studies have been carried out on 7 patients. The methodology employed is summarized in Table 1; the results are presented in Table 2. The method is a reversed phase HPLC separation adapted from that developed by Malspeis. Chromatograms of recovery standards, plasma standards and a patient sample are shown in Chart #1. The patient illustrated (#8, who received 200mg/m2/day merbarone) developed 2 putative metabolites, one of which is illustrated in 1c. The 4.5min and 10.7min peaks are, respectively merbarone, and the internal standard, pentothal.

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CLINICAL STUDIES

Steady state plasma levels were reached between 24 and 48 hours (see Chart #2); in 6 of the 7 patients the drug's disappearance from plasma fit well to a 2 compartment model. The Total Body Clearance was dose-independent over the dosage-range studied.

Although considerable variability between patients was observed in regard to rates of clearance, the ClTB in all of these patients was significantly slower than that observed in dogs i.e. 7.7 to 21.7 versus 34 to 149. In consequence, although the dosage rate we have studied is well below that producing toxicity in dogs (TDL 720mg/m2/day), the total AUC exposure of the patients at the 200mg/m2/day level is approaching that which produced toxic effects in dogs. Although toxicity has not been clinically impressive at the 200mg/m2 level, the available pharmacokinetic observations suggest that further escalations should be taken cautiously.

TABLE 1

MEASUREMENT OF MERBARONE

PROTEIN PRECIPITATION: 5:1 ADDITION METHANOL/DMSO 85/15

RECOVERY = 100% (CV = 4%)

REVERSE PHASE HPLC

-- C_{18} NOVAPAK STEEL COLUMN 15CM, 5µ PARTICLE

-- GRADIENT ELUTION (CONVEX) 40% —— 80% MEOH (15 MIN)

-- INTERNAL STANDARD: SODIUM PENTOTHAL

-- U.V. DETECTION 300MM

-- LIMIT OF DETECTION 0.5µG/ML, PRECISION CV = 3%

TABLE 2

PHARMACOKINETIC PARAMETERS DERIVED FROM EXPONENTIAL ANALYSIS OF THE CONCENTRATION x TIME DATA FOR MERBARONE

PATIENT #	DOSE MG/M2	CPSS µG/ML	AUC µG·HR/ML 0-∞	CLTB ML/MIN/M2	MRT HR	VDSS L/M2	α½ HR	β½ HR
1	100	3.2	385	21.7	10.1	13.1	0.98	8.4
2	100	4.8	567	14.7	11.8	10.4	0.20	11.4
3	100	9.1	1078	7.7	9.2	4.3	1.0	7.5
4	150	6.7	815	15.3	20.5	18.8	5.8	37.4
5	150	10.3	1301	9.6	65.7	37.9	7.9	132
7	200	9.0	1046	15.9	6.4	6.1	4.4	----
8	200	16.7	2003	8.3	16.2	8.1	0.27	14.2

AUC 0-∞ CORRECTED FOR INFUSION TIME.

NONLIN84 #10: A TWO-COMPARTMENT MODEL WITH CONSTANT I.V. INPUT, FIRST ORDER OUTPUT.
# 2: A ONE-COMPARTMENT MODEL WITH CONSTANT I.V. INPUT, FIRST ORDER OUTPUT.

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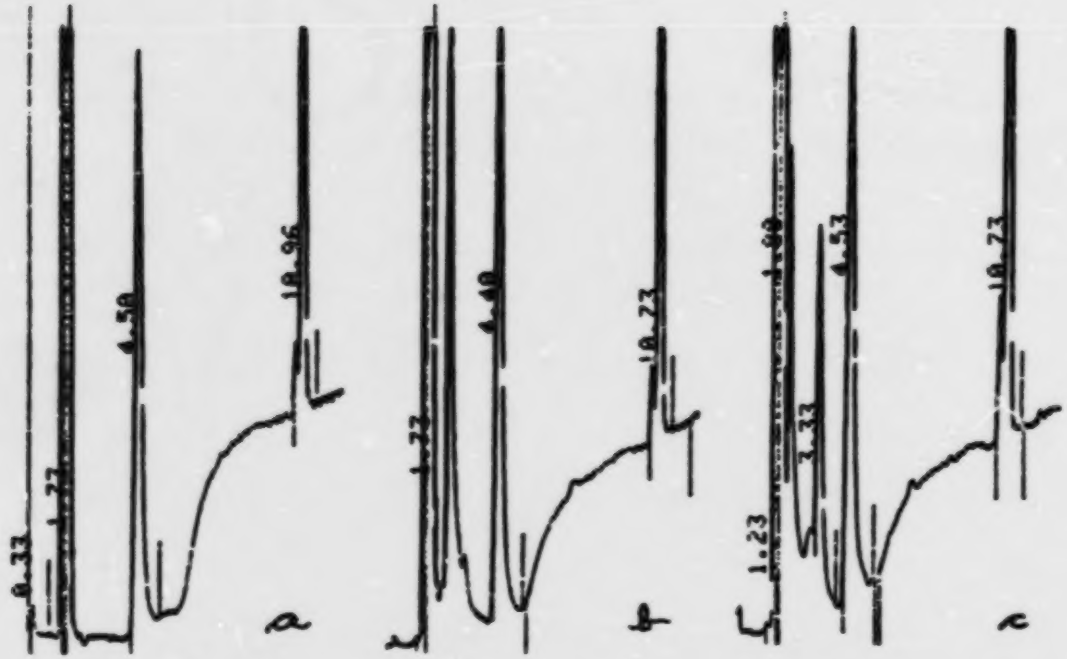

A: RECOVERY STANDARD 5µG/ML
B: PLASMA STANDARD 5µG/ML
C: PATIENT #8: PLASMA SAMPLE

CHART 1

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MERB PT 4       150MG/M2/DAY X5DAYS
NONLIN84 NONLINEAR ESTIMATION PROGRAM

FUNCTION  1
PLOT OF X VS. OBSERVED Y AND CALCULATED Y

*** ARE CALCULATED POINTS, 000 ARE OBSERVED POINTS
   7.910       I
   7.411       I                                0
   6.913       I                        0              0
   6.414       I                      *****************0
   5.915       I          0 0**********   0      0     0
   5.417       I          ***    0                     *
   4.918       I        **                              0
   4.419       I       *                                0
   3.921       I      *                                 0
   3.422       I      *                                  0
   2.923       I     *                                   *
   2.425       I     0                                    *
   1.926       I    0                                      *
   1.427       I    *                                       **0
  0.9287       I    0                                        ***0
  0.4300       I    0                                            ****0
                    +         +         +         +         +         +
                  1.000     33.93     66.87     99.80     132.7     165.7
                                        X
```

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CHART 2

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PHASE I TRIAL OF HMBA BY 10-DAY CONTINUOUS INFUSION

Characteristics of 11 Patients

AGE:	
RANGE	38 - 68
MEDIAN	56
PERFORMANCE STATUS:	
60	1 (9.1%)
70	3 (27.3%)
90	7 (63.6%)
SEX:	
MALE	8 (72.7%)
FEMALE	3 (27.3%)
PRIOR THERAPY:	
NONE	1 (9.1%)
CHEMOTHERAPY	3 (27.3%)
RADIATION THERAPY	0 (0%)
CHEMOTHERAPY & RADIATION THERAPY	7 (63.6%)
HISTOLOGIC DIAGNOSIS:	
ADENO CA	6 (54.5%)
EPIDERMOID CA	2 (18.2%)
LARGE CELL	2 (18.2%)
MELANOMA	1 (9.1%)

HEMATOPOIETIC TOXICITY - ALL COURSES

		WBC			Platelet		
Dose	**# of Courses**	**Median Nadir**	**(Day)**	**Range**	**Median Nadir**	**(Day)**	**Range**
12 gm/m^2/day	13	4.4	(16)	2.1-7.7	146	(15)	53-245
16 gm/m^2/day	10	4.7	(15)	3.6-9.3	127	(15)	70-250

HEMATOPOIETIC TOXICITY - INITIAL COURSE

		WBC			Platelet		
Dose	**# of Patients**	**Median Nadir**	**(Day)**	**Range**	**Median Nadir**	**(Day)**	**Range**
12 gm/m^2/day	6	5.8	(15)	3.1-7.7	129	(15)	53-245
16 mg/m^2/day	5	4.8	(14)	3.6-9.3	114	(15)	77-226

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HMBA PHARMACOKINETICS

Internal Standard: PMBA

Methanol Elution from C-18 Sep-Paks

0.75mm x 30m glass capillary column at 240°C
Supelco SPB-35

Injection Temp 300°C - 2 ul injection volume

NPD detector at 300°C

Carrier gas: Helium

Linear Range 0.01 - 10.0mM

C.V. 7.5%

Walsh TD and Fanucchi MP Journal of Chromatography In Press

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HMBA Pharmacokinetics - Steady state plasma concentrations

PATIENT #	Dosage gm/m2/day	No Samples	Mean HMBA* mM	Observed Range
867681	12	11	0.27 ± 0.11	0.18 to 0.35
834708	12	11	0.52 ± 0.11	0.36 to 0.61
834708∤	12	7	0.73 ± 0.05	0.72 to 0.80
271832	12	17	0.24 ± 0.21	0.16 to 1.8
868667	12	18	0.88 ± 0.05	0.44 to 1.9
853667	12	15	0.30 ± 0.1	0.21 to 0.49
838358	12	18	0.48 ± 0.17	0.25 to 1.5
838358**	12	4	0.66 ± 0.38	0.33 to 1.9
838358**	12	6	0.74 ± 0.69	0.3 to 2.1
867681≠	16	9	0.60 ± 0.05	0.53 to 0.72
867448	16	8	0.76 ± 0.11	0.6 to 0.92

---

*± Standard Deviation

** Repeated Study

∤ Repeated Study

≠ Dose Escalation

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HMBA Phase I by 10-Day Continuous Infusion

	Dose gm/m^2/day	CL$_{cr}$ ml/min	Mean HMBA mM	Platelet Nadir x10^3
867681	12	72	0.27	245
834708	12	64	0.52	175
271832	12	118	0.24	53
868667	12	96	0.88	95
853667	12	124	0.30	140
838358	12	76	0.48	118
867448	16	68	0.76	114

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Johns Hopkins
July 1986

PHASE I STUDY OF HMBA
THE JOHNS HOPKINS ONCOLOGY CENTER

David S. Ettinger, M.D.
Eric K. Rowinsky, M.D.
Ross C. Donehower, M.D.

In our initial phase I study of HMBA, the drug was given as a continuous five-day infusion every three weeks to patients with advanced cancer. Dose-limiting toxicities included renal insufficiency, a hyperchloremic metabolic acidemia/acidosis, and central nervous system toxicities manifested by agitation and delirium. Moderate myelosuppression, mucositis, nausea and vomiting were also observed.

Based on our initial trial, the MTD and recommended phase II doses for HMBA administered on the above schedule are 33.6 and 24 gm/M^2/day respectively.

HMBA concentrations in plasma and urine were measured by gas chromatography using octamethylene bisacetamide (OMBA) as an internal standard. HMBA concentrations were determined by comparing peak area ratios between HMBA and the OMBA internal standard.

Since steady-state HMBA levels at these doses were in the range of 1-2 mM (Table 1), only approaching the lower limit demonstrated for in vitro differentiating effectiveness, and because of evidence suggesting that the exposure period is an important variable in the induction of differentiation, additional studies examining longer period of infusion were warranted.

After completion of the above phase I study of HMBA, the schedule of administration from a continuous infusion daily x 5 of a 21-day cycle was changed to a continuous infusion daily x 10 of a 28-day cycle. Our plans

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were to start with a dose of 15.8 gm/M^2/day, since we saw little toxicity at this dose level. If tolerated, we were to increase the dose to 20.0 gm/M^2/day followed by 24.0 gm/M^2/day, if appropriate. Thus far, eight patients received ten courses of HMBA.

Patient characteristics are shown in Table 2. The number of patients and courses for each dose escalation is shown in Table 3. The toxicity for both dose levels appears in Table 4. At the 15.8 gm/M^2/day dosage level, one of five courses had nausea and vomiting, thrombocytopenia, acidemia/acidosis, and skin rash associated with it. The details of the skin rash will be discussed later. At the 20 gm/M^2/day dosage level, two of five patients are still receiving their infusions. Of the three evaluable courses, grade 2, 3 and 4 thrombocytopenia have been seen. Table 5 demonstrates the effect of HMBA on the platelet count. It should be noted that the nadir platelet count following HMBA administration occurs approximately on day 15, with the day of recovery, approximately on day 21. Leukopenia has also been seen at the 20 gm/M^2/day dosage level.

In regard to the skin rash seen at the 15.8 gm/M^2/day dosage level, it occurred in a 65-year old white female with a leiomyosarcoma of the jejunum with metastases to the liver. Past treatment included resection of the jejunum, doxorubicin and trimetrexate. On day 12 following the first course of HMBA, the patient developed a periorbital and malar erythematous rash which intensified until day 15 and resolved by day 22 without desquamation. An ANA titer was positive at 1:40. During the second course of HMBA, the patient on day 3 developed purpura on the ankles which progressed proximally to the thighs by day 9. She also redeveloped the facial skin rash on day 10 which resolved by day 22. The infusion was discontinued on day 9 with resolution of the extremity skin rash by day 22. There was no evidence of extradermal vasculitis. An ANA titer was positive at 1:80 while serum C_3, C_4, immune complexes were normal.

A biopsy of the skin lesion revealed a leukocytoclastic vasculitis with perivascular deposition of IgA and C_3, revealing a drug-induced vasculitis.

Thus far, no antitumor response has been seen following the administration of HMBA.

Table 6 summarizes the effects of HMBA when administered as a ten-day infusion.

Mean steady-state HMBA plasma concentrations (Css) and clearances from several patients treated at both dose levels are shown in Table 7.

In summary, if the MTD turns out to be 20 gm/M^2/d as a continuous 10-day infusion every 28 days, then we would recommend returning to the continuous 5-day infusion every 21 days for phase II studies, for example in myelodysplastic syndromes.

HMBA
T84-0447
Johns Hopkins
July 1986

TABLE 1

HMBA STEADY-STATE CONCENTRATION CLEARANCE

Dose (g/m²/d)	Mean Steady-State HMBA Concentration (range) (mM)	Mean Systemic Clearance (range) (cc/min/m²)	Mean Renal Clearance (range) (cc/min/m²)	Mean Systemic/Renal Clearance
15.0	0.70 (0.54-0.97)	90 (57-102)	45 (39-57)	0.51
15.0	0.71 (0.59-0.85)	79 (65-93)	-	-
15.0	1.50	37	6 (5-6)	0.15
15.0	0.61 (0.53-0.68)	91 (81-103)	42 (32-51)	0.44
15.0+	0.88 ± 0.10	74 ± 13	31 ± 13	0.37 ± 0.11
24.0	0.53 (0.49-0.56)	150 (140-170)	74 (41-91)	0.51
24.0	0.47 (0.32-0.60)	185 (139-260)	-	-
24.0	0.62 (0.60-0.63)	136 (132-139)	72 (60-89)	0.59
24.0	0.69 (0.63-0.76)	121 (110-132)	52 (43-61)	0.45
24.0	1.34 (0.82-1.68)	66 (53-102)	18 (15-20)	0.33
24.0*	1.90	44	7 (6-9)	0.32
24.0	0.44 (0.33-0.52)	193 (160-203)	91 (34-98)	0.51
24.0	0.73 (0.64-0.88)	115 (95-130)	83 (57-107)	0.70
24.0	0.88 (0.84-0.94)	95 (88-99)	56 (44-66)	0.51
24.0	2.20 (1.82-2.58)	36 (35-46)	13 (10-17)	0.37
24.0	1.29 (1.04-1.44)	65 (51-80)	24 (11-39)	0.43
24.0	1.35 (1.02-1.54)	63 (54-82)	35 (10-50)	0.55
24.0+	0.98 ± 0.16	107 ± 16	48 ± 9	0.47 ± 0.03
33.0	1.10 (1.00-1.16)	76 (71-83)	47 (38-56)	0.61
33.0	1.36 (1.11-1.46)	87 (73-105)	55 (40-69)	0.62
33.0	1.72 (1.37-2.05)	69 (62-85)	-	-
33.0	2.09 (1.70-2.38)	57 (49-69)	19 (15-26)	0.37
33.0*	1.66 (1.25-2.33)	75 (50-93)	-	-
33.0+	1.58 ± 0.17	73 ± 6	60 ± 11	0.53 ± 0.10

* - Courses associated with central nervous system toxicity.
+ - mean ± standard error.

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Table 2

HMBA (X 10 DAYS) PATIENT CHARACTERISTICS

Number of Patients Entered		8
Number of Courses (Infusion completed)		10 (8)
Sex Ratio (M:F)		4:4
Median Age (Range)		61 (53-69)
Performance Status		0 - 3 1 - 1 2 - 3 3 - 1
Prior Therapy	Chemotherapy	3
	Radiotherapy	2
	Both	3
	None	0
Tumor Types:	Colon	2
	Lung	2
	Sarcoma (small intestine)	1
	Ovary	1
	Bile Duct	1
	Endometrial	1

Table 3

HMBA (X 10 DAYS) DOSE ESCALATION

Daily Dose x 10 (gm/M^2)	No. of Patients	No. of Courses (infusions completed)
15.8	3(3)	5(5)
20	5(3)	5(3)*

* 2 additional courses are in progress as of 7/8/86

Table 4

HMBA (X 10 DAYS) TOXICITY

Dose (gm/M^2/d)	No. of Courses Infusion Completed	Toxicity Grade	Nausea and Vomiting	Thrombo-cytopenia	Leuko-penia	Skin	Renal	Acidemia/ Acidosis+
15.8	5	0	4	4	5	3	5	1/5
		1	0	0	0	1	0	
		2	1	1	0	1	0	
		3	0	0	0	0	0	
20	3*	0	3	0	0	3	2	0/3
		1	0	0	2	0	1	
		2	0	1	0	0	0	
		3	0	1	0	0	0	
		4	0	1	1	0	0	

* 2 additional courses are in progress as of 7/8/86

+ No. of courses with serum bicarbonate concentration ≤ 20 meq/L

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Table 5

EFFECT OF HMBA ON PLATELET COUNT

Dose (gm/M^2/D)	Pretreatment Platelet Count (x 10^3)	Nadir Platelet Count (x 10^3)(day)	Day of Recovery
15.8	224	63 (d18)	Day 25
15.8	346	207 (d 9)	Day 22
15.8	375	141 (d11)	Day 17
15.8	384	137 (d15)	Day 22
15.8	560	214 (d15)	Day 20
20	225	63 (d15)	--
20	320	19 (d17)	--
20	333	36 (d13)	--

Table 6

HMBA (X 10 DAYS) SUMMARY OF EFFECTS

Hematologic:
- Thrombocytopenia - nadir around d15, resolves by d21 (3/3 at 20 gm/M^2/d)
- Leukopenia (1/3 at 20 gm/M^2/d)

Renal:
- ↑ BUN, Cr during infusion, resolves quickly after infusion (1/2 at 20 gm/M^2/d - Cr=1.8 mg%)
- Acidemia/Acidosis, (1/5 at 15.8 gm/M^2/D, HCO_3/Cl $^{25}/106$ --> $^{20}/107$)

Neurologic:
- No clinical CNS toxicity
- No change in daily mini-mental exams
- 4/6 post-treatment EEGs - mild diffuse disturbance of cerebral function

Skin:
- Vasculitis

Other:
- Nausea and Vomiting (1)
- Anorexia (1)
- Malaise (1)
- Leukocytosis (6/7 courses)

Table 7

HMBA (X 10 DAYS) STEADY-STATE CONCENTRATION CLEARANCE

Dose ($gm/M^2/d$)	Mean Steady-State HMBA Concentration (range) (mM)	Mean Systemic Clearance (range) ($cc/min/M^2$)	Mean Renal Clearance (range) ($cc/min/M^2$)	Mean Renal/Systemic Clearance
15.8	0.63 (0.48-0.75)	89 (73-114)	44 (35- 61)	0.50
15.8	0.35 (0.26-0.48)	164 (114-211)	79 (46-109)	0.50
15.8	0.23 (0.15-0.27)	246 (203-274)		
15.8	0.45 (0.33-0.73)	126 (107-166)		
15.8	0.48 (0.36-0.43)	140 (128-152)		
15.8 (mean ± s.e.)	0.43 ± 0.17	153 ± 4		
20.0	0.31 (0.25-0.36)	229 (193-257)		
20.0	1.72 (1.02-2.28)	43 (30- 64)		
20.0 (mean ± s.e.)	1.00 ± 0.70	136 ± 8		

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Trimetrexate
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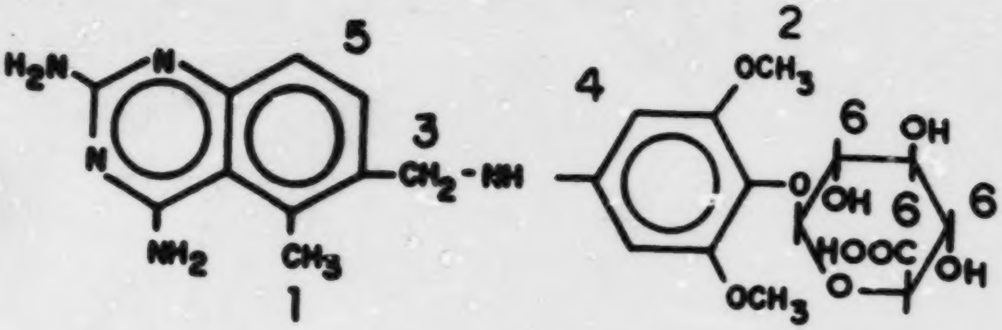
Vermont
July 1986


5
2
4
3
6
6
6
1
H2N
N
N
NH2
CH3
CH2-NH
OCH3
OCH3
OH
OH
HOOC
OH
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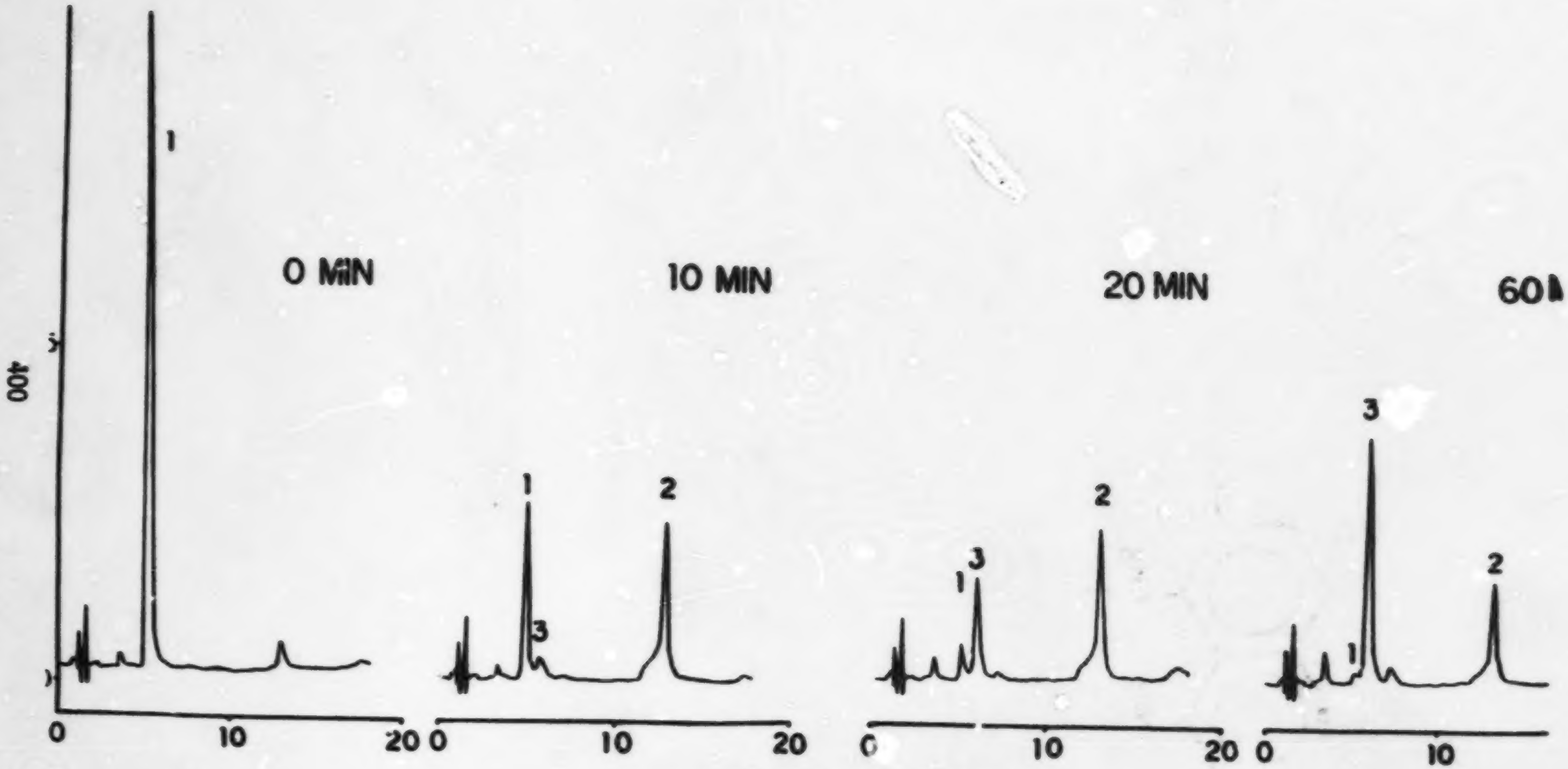

CHANGES IN CHROMATOGRAM (MOUSE METABOLITE 1)
PRODUCED BY GLUCURONIDASE TREATMENT

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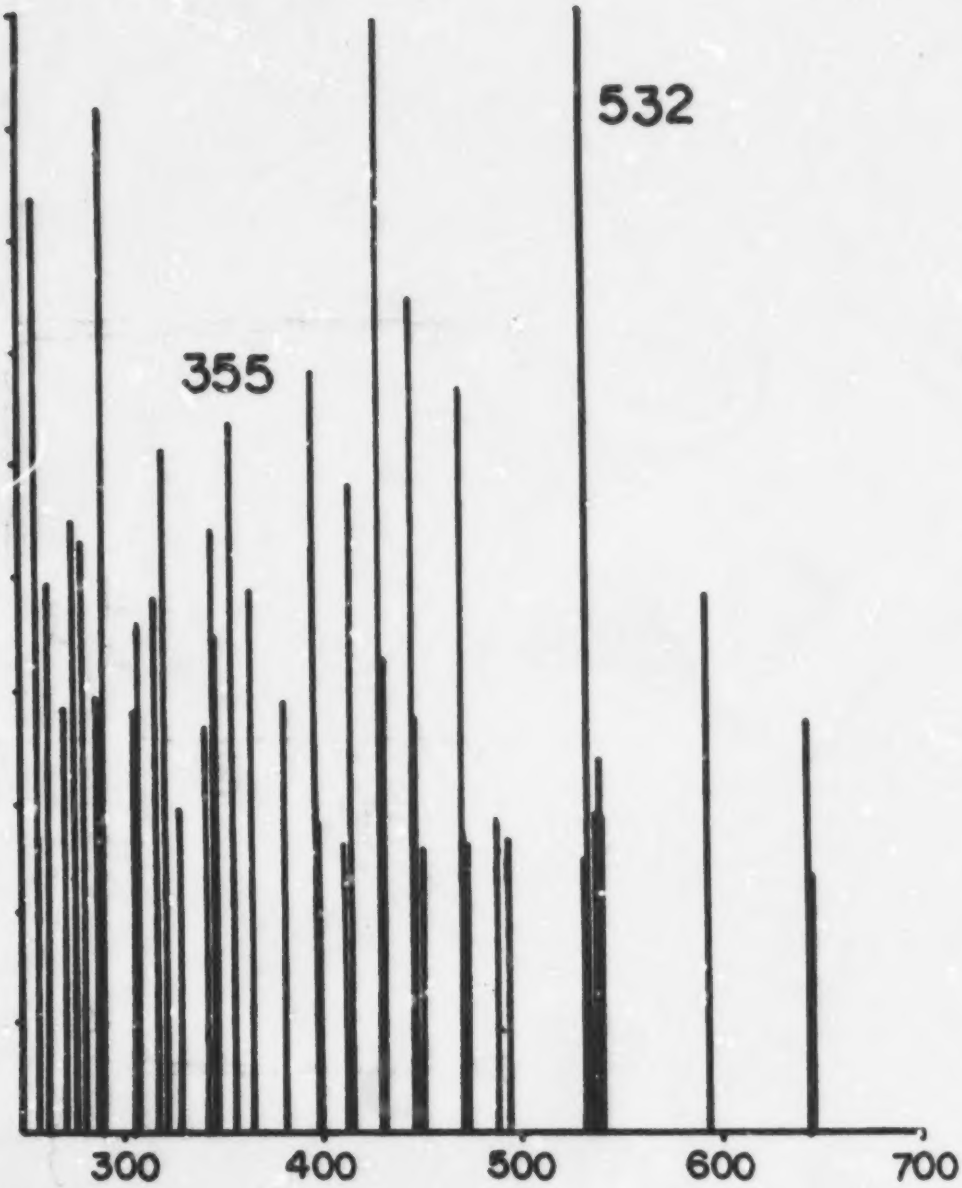

FAB MASS SPECTRUM*

(MOUSE METABOLITE: THIOGLYCEROL)

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*G. McCLUSKEY AND E. BERMAN (WARNER-LAMBERT)

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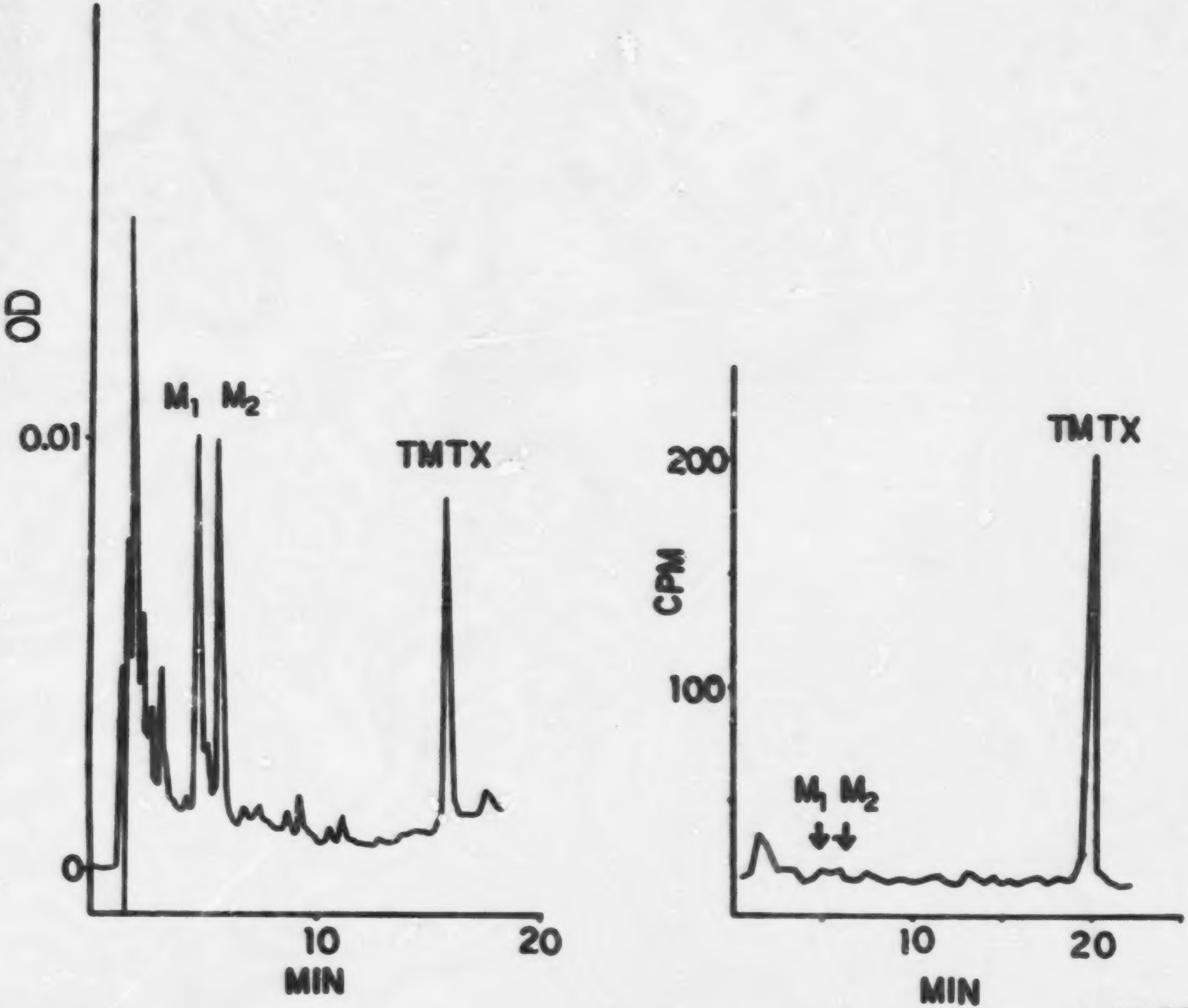

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HPLC PATIENT URINE SAMPLE

CONCLUSIONS

1. TMTX IS CONVERTED, IN HUMANS, TO TWO METABOLITES (MET 1, MET 2).

2. MET 1 IS IDENTICAL IN CHROMATOGRAPHIC BEHAVIOR TO THE MAJOR METABOLITE FORMED IN MICE.

3. MET 1 IS FORMED BY LOSS OF THE METHYL GROUP AT POSITION 4' WITH SUBSEQUENT GLUCURONIDE FORMATION.

4. 4-O'-DEMETHYLATION ALSO IS INVOLVED IN FORMATION OF MET 2.

5. MET 1 IS ELIMINATED, IN THE ISOLATED PERFUSED RAT LIVER, BY BILIARY EXCRETION.

Trimetrexate
T84-0268
Johns Hopkins
July 1986

PHASE I STUDY OF TRIMETREXATE ON A DAILY X 5 SCHEDULE

The Johns Hopkins Oncology Center
Baltimore, Maryland 21205

Louise B. Grochow, M.D.
Ross C. Donehower, M.D.
David S. Ettinger, M.D.

We have previously completed a phase I study of this lipid-soluble quinazoline antifolate using a single bolus dose of drug repeated every 3 weeks. The recommended phase II dose for this schedule was 220 mg/M^2 and the major toxicities were hematologic, gastrointestinal, and cutaneous.

The preclinical information which was available about the schedule dependency of this drug suggested that multiple dosing schedules were superior to the single dose. Therefore, two studies of the daily x 5 schedules and other multiple dose schedules have been evaluated.

In this study, 35 patients have been treated. The patient characteristics are shown in Table 1. The number of the patients and courses which have been evaluable at each dose level are shown in Table 2.

The non-hematologic toxicity of TMTX is shown in table 3. Other than a small number of patients who had either significant discomfort from a pruritic skin rash or had grade 2 or higher mucositis, these toxicities were not of great significance and did not limit dose escalation. The mucositis tended to occur in patients who had coincident hematologic toxicity.

The hematologic toxicity which has limited further dose escalation and represents the major clinical problem encountered is detailed in Table 4-7. Table 4 shows the number of courses at each dose level associated with leucopenia of varying degrees. The counts listed above the top of this table correspond to ECOG grades 0-4. All courses are included whether they were first or subsequent courses of drug and regardless of prior treatment status. Grade 4 toxicity occurred even at the standing dose and illustrates the difficulties encountered defining an appropriate dose for further clinical trials with this drug. WBC nadir typically occurred on day 8-10 and recovered completely by day 15-18. Table 5 demonstrated the data for platelet toxicity in the same fashion. Thrombocytopenia was not as common as leucopenia, in general was not as severe, and occurred typically on day 15.

A major determinant of the hematologic toxicity which was observed was prior treatment status of the patients. Patients who had received prior RT (in this study principally pelvic RT) or mitomycin C were predisposed to severe toxicity. Shown in Table 6 and 7 are the grades of hematologic toxicity encountered by patients receiving their first dose of TMTX at 9.1, 10.9, or 13.1 mg/M^2, and whether or not they had received prior RT or mitomycin. The asterisks indicated the 2 patients who are included in this analysis who had mitomycin but not RT.

No responses have been seen. Two of the 23 colon cancer patients have had decreases in CEA noted which persisted for 2 and 6+ months. Only 5 of these patients had not received prior therapy.

Pharmacokinetic studies also accompanied this protocol, using a competitive dihydrofolate reductase binding (DHFR) assay. Our study of the kinetics of TMTX after a single i.v. bolus in our prior study demonstrated the plasma disappearance to be biphasic with initial and terminal half lines of 25.2 min and 7.9 hours, respectively. VD$_{ss}$, was 26.0 liters, Clearance was 37 ml/min/M^2. In the current study, samples were obtained from all patients prior to and 1 hour after each dose on their first course of therapy. This time point was chosen because it had been shown to consistently fall on the terminal half line portion of the disappearance curve. These values were fitted to a one compartment model using PCNONLIN and a model for repeated i.v. bolus. The equation is shown in Table 8. The results for the first 31 patients are shown in Table 9.

Correlations of various parameters with toxicity were sought. Both total AUC and mean 1 hour post-infusion concentration correlated with leucopenia as measured by the numerical change in WBC's from pretreatment to nadir. This correlation may be useful as a clinical predictor of severe toxicity if our further studies support this relationship.

Based on this study recommended phase II doses would be 7.6 mg/M^2 for previously treated patients and 13.1 mg/M^2 for previously untreated patients. Because of the variability of individual patient tolerance, escalation schemes as well as provisions for de-escalation should be built into these studies. In consultation with NCI personnel these recommendations have been formalized as 8 and 12 mg/M^2, respectively.

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July 1986

TABLE 1

TRIMETREXATE/DOSE ESCALATIONS

Dose (mg/M^2)	No. Patients Evaluable	No. of Courses Evaluable
7.6	10	22
9.1	13	21
10.9	12	20
13.1	11	18
15.7	2	2
18.8	1	1

TABLE 2

TRIMETREXATE PATIENT CHARACTERISTICS

No. of Patients Entered:		35		
No. of Courses (Evaluable):		83(81)		
Sex:	Male	19		
	Female	16		
Age:	Median	60		
	Range	30-74		
Performance Status:	Median	1		
	Range	0-2		
Prior Therapy:	Chemotherapy	14		
	Radiotherapy	2		
	Both	12		
	None	7		
Malignancy:	Colon:	23	Ampulla of Vater	1
	Rectum	3	Gastric	1
	Cervix	2	Lung	1
	Breast	1	Kidney	1
	Small Bowel	1	Pancreas	1

TABLE 3

TRIMETREXATE TOXICITY

Dose mg/M²	# Eval. Courses	ECOG Grade	N & V	Malaise	Mucositis	Alopecia	Skin
7.6	22	0	19	16	16	17	19
		1	0	5	4	3	3
		2	2	1	2	0	0
		3	1	0	0	0	0
9.1	21	0	19	13	15	20	19
		1	1	6	2	0	0
		2	1	1	2	0	2
		3	0	1	2	0	1
		4					
10.9	20	0	17	11	12	18	15
		1	1	7	5	1	4
		2	1	0	1	1	1
		3	1	2	2	0	0
13.1	18	0	14	10	14	17	16
		1	2	5	1	1	0
		2	0	3	3	0	2
		3	0	0	0	0	0
		4					
15.7	2	0	2	0	0	2	2
		1	0	2	2	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4					
18.8	1	0	1	0	1	1	1
		1	0	1	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4					

TABLE 4

HEMATOLOGIC TOXICITY (WBC'S)

Dose	N:	>4.5	3.0-4.5	2.0-3.0	1.0-2.0	<1.0
7.6	22	7	3	6	2	4
9.1	21	11	2	2	2	4
10.9	20	5	3	6	2	4
13.1	18	7	7	0	2	2
15.7	2	1	0	0	1	0
18.8	1	1	0	0	0	0

TABLE 5
HEMATOLOGIC TOXICITY (PLATELETS)

Dose	N:	>130	90--130	50-90	25-50	≤25
7.6	22	13	2	5	1	1
9.1	21	14	2	1	3	1
10.9	20	13	2	1	0	4
13.1	18	13	2	1	1	1
15.7	2	1	0	0	1	0
18.8	1	1	0	0	0	0

TABLE 6
EFFECT OF PRIOR TREATMENT ON HEMATOLOGIC TOXICITY
(WBC's)

Dose	N:	RT	0	1	2	3	4
9.1	12	+	2	0	0	1	6
		-	2	0	1	0	0
10.9	9	+	0	0	0	2	4
		-	2	0	1	0	0
13.1	8	+	0	0	0	0	2
		-	3	1	1	0	1

TABLE 7
EFFECT OF PRIOR TREATMENT ON HEMATOLOGIC TOXICITY

(PLATELETS)

			(Grade)				
Dose	N:	Rt	0	1	2	3	4
9.1	12	+	3	1	1	3	1
		-	3	0	0	0	0
10.9	9	+	2	0	0	0	4
		-	2	0	0	0	1
13.1	8	+	0	0	1	0	1
		-	5	1	0	0	0

TABLE 8

$$C_p = \frac{\text{Dose}}{\text{Volume}} \text{ X } \frac{e^{-kt} \text{ x } (1 - e^{-kTN})}{(1 - e^{-kT})}$$

Trimetrexate
T84-0268
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July 1986 TABLE 9

Trimetrexate
Daily x 5

#	dose	VOL L	K10 min-1	T 1/2 mins	CL L/M2	AUC uM*min	Css(1hr) uM	Css(24h) uM
45	20.6	20.98	0.0028	248	0.059	351	0.85	0.02
46	20.6	16.51	0.0027	260	0.044	469	1.09	0.03
47	20.6	20.51	0.0026	267	0.053	387	0.88	0.02
48	20.6	27.54	0.0023	304	0.063	328	0.68	0.03
49	20.6	22.69	0.0022	318	0.049	416	0.83	0.04
50	20.6	23.22	0.0046	152	0.106	195	0.68	.00
52	24.6	16.08	0.0025	283	0.039	625	1.36	0.05
53	24.6	10.78	0.0023	297	0.025	978	2.06	0.08
54	24.6	10.25	0.0024	293	0.024	1013	2.15	0.08
56	24.6	11.25	0.0023	300	0.026	947	1.98	0.08
57	24.6	6.12	0.0027	257	0.017	1491	3.49	0.08
58	24.6	14.57	0.0021	331	0.030	807	1.57	0.09
59	24.6	8.89	0.0021	327	0.019	1307	2.56	0.14
60	24.6	7.70	0.0025	281	0.019	1295	2.84	0.09
61	24.6	6.38	0.0024	284	0.016	1577	3.43	0.12
62	24.6	5.54	0.0022	318	0.012	2039	4.08	0.20
63	24.6	11.21	0.0013	528	0.015	1673	2.39	0.39
64	24.6	12.02	0.0021	325	0.026	960	1.89	0.10
65	29.5	9.08	0.0025	273	0.023	1277	2.86	0.09
66	29.5	12.97	0.0021	339	0.027	1112	2.12	0.13
67	29.5	13.85	0.0020	339	0.028	1043	1.99	0.12
68	29.5	2.97	0.0031	224	0.009	3213	8.36	0.12
69	29.5	6.90	0.0028	247	0.019	1526	3.68	0.08
70	29.5	15.20	0.0025	273	0.039	764	1.71	0.05
71	29.5	19.63	0.0021	334	0.041	724	1.40	0.08
72	35.5	14.61	0.0023	302	0.034	1058	2.20	0.09
73	35.5	23.29	0.0021	333	0.048	732	1.42	0.08
74	35.5	14.46	0.0024	286	0.035	1012	2.19	0.08
75	35.5	7.22	0.0020	354	0.014	2512	4.65	0.31
76	35.5	12.36	0.0021	325	0.026	1347	2.65	0.14
	Mean	13.49	0.0024	300.1	0.033	1106		
	Std.d.	6.07	0.0005	58.9	0.019	646		

Pharmacokinetic Summary

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Figure 1

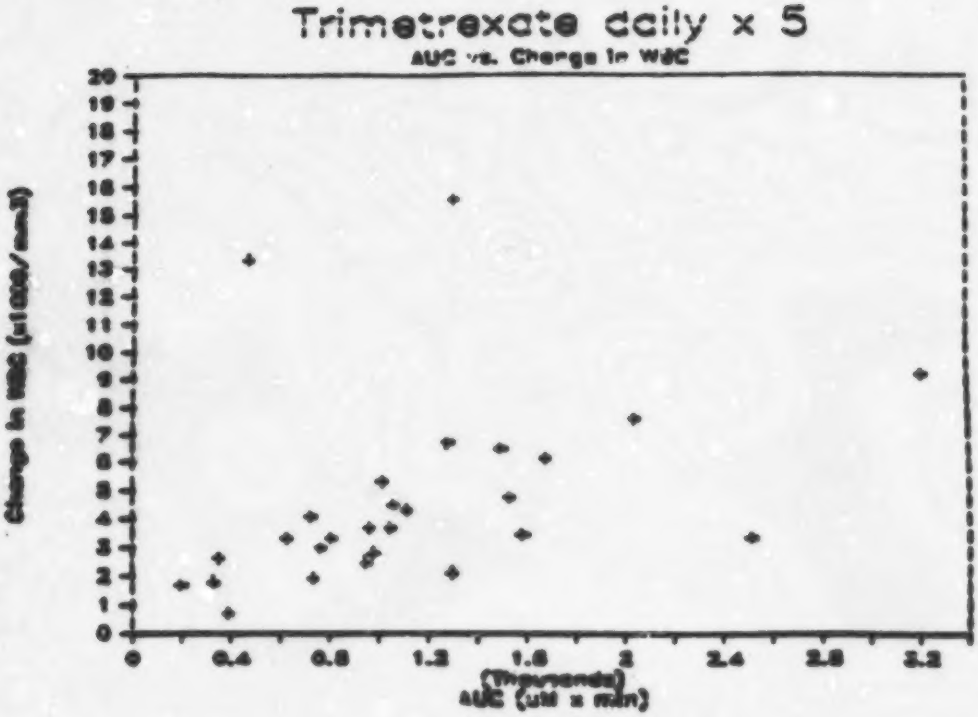
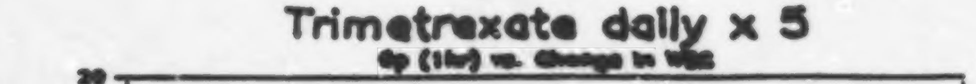


Figure 2



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SR2508
RTOG-83-03
DFCC
July 1986

Phase I trial of the hypoxic cell
radiosensitizer SR 2508- presentation
to the NCI Phase I meeting July 9, 1986
Bethesda, MD, C. Norman Coleman, M.D.

DEVELOPMENT OF SR 2508

1. SR 2508 is a 2-nitroimidazole, in the same class as Misonidazole.

2. Miso had a dose limiting neurotoxicity, both peripheral and central.

3. SR 2508 is a less lipophilic analog, designed to be less neurotoxic than Miso.

TRIAL DESIGN

1. Drug given by rapid i.v. infusion, 30-60 minutes before radiation.

2. Determine maximum tolerated cumulative dose, ultimately over 5-6 weeks to accompany curative radiotherapy.

3. Some information derived regarding MTD for single dose.

References:
1. Coleman, CN, Urtasun, RC, Wasserman, TH, Hancock, S, Harris, JW, Halsey, J, Hirst, UK: Initial report of the phase I trial of the hypoxic cell radiosensitizer SR 2508. Int J Radiat Oncol Biol Phys 10: 1749-1754, 1984.

2. Coleman, CN, Wasserman, TH, Urtasun, RC, Halsey, J, Hirst, UK, Hancock, S, Phillips, TL: Phase I trial of the hypoxic cell radiosensitizer SR 2508: The results of the five to six week schedule. Int J Radiat Oncol Biol Phys (in press) 1986.

3. Coleman, CN, Halsey, J, Cox, RS, Hirst, UK, Blaschke, T, Howes, AE, Wasserman, TH, Urtasun, RC, Pajak, T, Hancock, S, Phillips, JTL: Prediction of the neurotoxicity of the hypoxic cell radiosensitizer SR 2508 from the pharmacokinetic profile. Cancer Res (accepted for publication).

SR2508
RTOG-83-03
DFCC
July 1986

Mean Plasma Concentration of SR 2508 (µg/ml)

Dose size (g/m^2)	# pts.	peak	1 hr	2 hr	4 hr	8hr
1.3	3	160	58	40	28	16
1.7	9	188	74	49	35	21
2.0	42	239	78	58	37	21
2.3	4	277	112	70	51	28
2.7	3	291	127	93	85	33
3.0	1	360	132	·81	48	24
3.3	6	347	157	101	70	43
3.7	3	425	188	117	83	48

Table 3. Tumor biopsies SR-2508 (µg/ml)

	Infusion duration (min)	Time of tumor sample	SR-2508 (tumor)		SR-2508 (plasma)	Tumor/Plasma ratio %
Cervix ca normal mucosa 1.7 g/m^2	17	22″	87		138	63
		22″	94		138	68
Eyelid basal cell ca 3.7 g/m^2	4	10″	453		305	143
		20″	140		254	55
		30″	132		216	61
Bladder ca 3.3 g/m^2	12	18″	76		230	33
		28″	135		182	74
		38″	299		161	185
Fibroushistiocytoma abdomen 1 g/m^2	6	6″	33.9	7″	151	22
		8″	56.6	9″	129	44
		11″	54.2			
		15″	52.2	14″	83.5	62
		23″	38.05	30″	51.6	
		41″	28.6	45″	41.2	69
		53″	27.5			
		60″	33.0			

Table 1. Peripheral neuropathy, SR-2508 three week schedule

Peripheral neuropathy, per assigned step		Peripheral neuropathy, per actual dose administered	
Total dose planned g/m^2 on assigned step	No. patients with neuropathy/No. evaluable	Total dose received, g/m^2	No. patients with neuropathy/No. evaluable patients
11.7	0/2	≤17.0	0/17
15.3	0/4	18.0–20.7	0/4
18.0	0/3	21.6–24.3	2/5
20.7	0/4	26.4–27.0	2/4
24.3	1/3	29.6–29.7	3/5
27.0	1/3	31.9–32.1	2/2
29.7	4/6		
33.0	3/3		

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DRUG SCHEDULE AND NEUROTOXICITY

Total dose, g/m^2	Dose, g/m^2	# of weeks	# pts toxic	#evaluable
11.7	1.3	3	0	2
15.3	1.7	3	0	4
18.0	2	3	0	3
20.7	2.3	3	0	4
24.3	2.7	3	1	3
27	3	3	1	3
29.7	3.3	3	4	8
33	3.7	3	3	3
30	2	5	0	6
36	2	5	6	*10
40	2	5	8	8
40.8	1.7	8	5	8
36	2	6 split	4	5
34	2	6 split	2	7
36	2	6 every other day	5	**8

One of the evaluable patients had total dose of *35, **34 g/m^2

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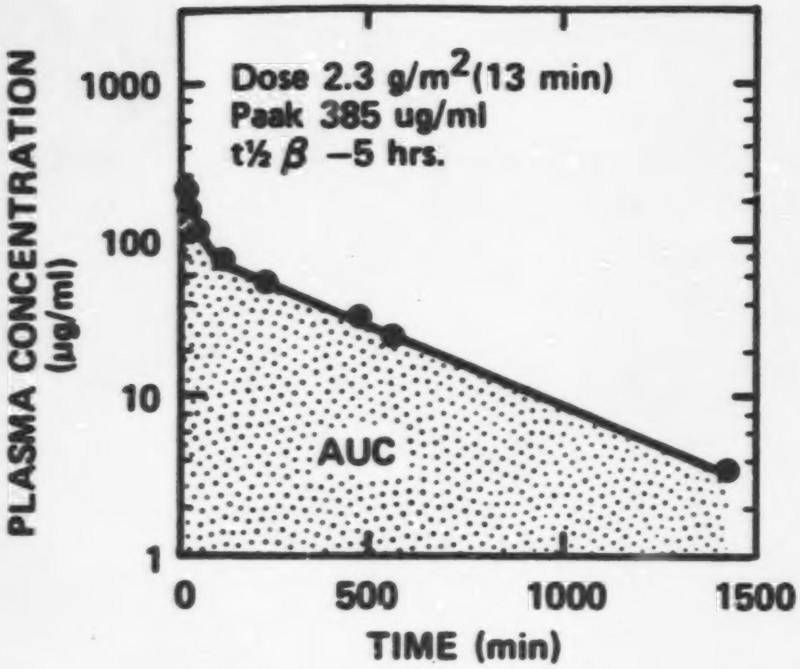
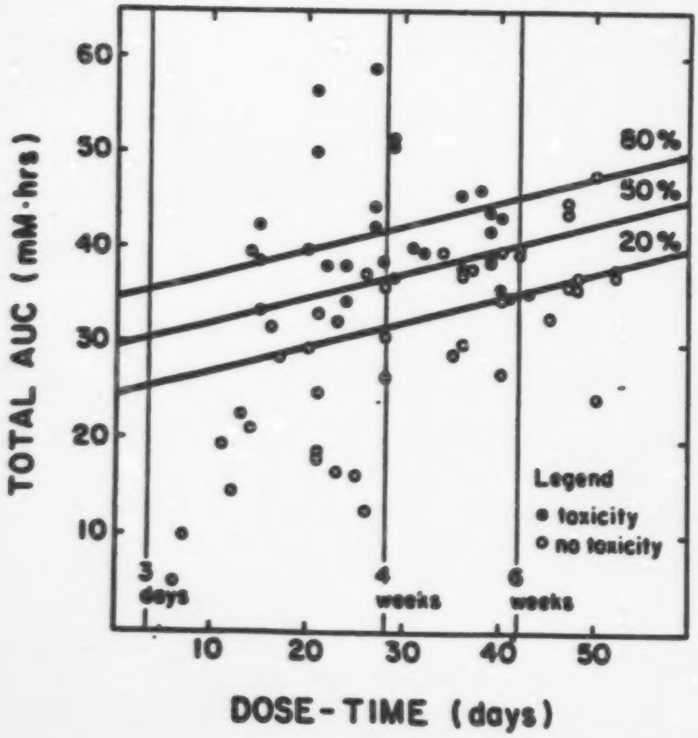

Dose 2.3 g/m2(13 min)
Paak 385 ug/ml
t½ β −5 hrs.
AUC
PLASMA CONCENTRATION (µg/ml)
1000
100
10
1
0
500
1000
1500
TIME (min)


TOTAL AUC (mM·hrs)
60
50
40
30
20
10
80%
50%
20%
Legend
● toxicity
○ no toxicity
3 days
4 weeks
6 weeks
10
20
30
40
50
DOSE - TIME (days)

SR2508
RTOG-83-03
DFCC
July 1986

GENERAL CONCLUSIONS (1)

1. SR 2508 is about 1/3 as toxic as Miso. Dose-limiting toxicity is peripheral neuropathy; no central neuropathy seen.

2. A single dose of 2 g/m^2 will produce a tumor concentration of 100 µg/ml, which will produce an sensitizer enhancement ratio (SER) of 1.6 for the hypoxic cells.

3. The starting total dose for the 5 week schedule will be 34 g/m^2.

4. If the predictability of the neuropathy is verified, this will be used to determine dose.
5. Phase II and III trials under consideration for:
   - Head and neck cancer
   - Prostate cancer
   - Bladder cancer
   - Lung cancer
   - Esophageal cancer

6. Phase I trial as continuous infusion for use with brachytherapy.

OTHER POTENTIAL FOR THESE COMPOUNDS

1. Combination of sensitizers, SR 2508 + Ro-03-8799- different toxicities.

2. Dual function agents: RSU-1069.

3. Agents that are selectively toxic to hypoxic cells: SR 4233, RSU-1069.

4. Hypoxic cell imaging.

5. Chemo-modification.

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ROBERTS LABORATORIES, INC.
SR-2508
SCOPE OF CLINICAL PROGRAM

1. Head and neck squamous cell carcinoma study (SR-2508 Study No. 1).

2. Bladder carcinoma (T_3) study (SR-2508 Study No. 2).

3. SR-2508 tumor levels.

ROBERTS LABORATORIES, INC.
SR-2508
HEAD AND NECK CARCINOMA STUDY - PHASE II/III STUDY

-- Parallel program to NCI/RTOG study #85-27, employing essentially the same protocol.

-- 300-400 patients at 15-20 centers in Europe under the leadership of the Institut Gustave Roussy, Paris, France, who will provide statistical, SR-2508 assay and radiotherapy equipment quality assurance support. F.D.A. guidelines will be followed and field monitoring will be done by Roberts Laboratories.

ROBERTS LABORATORIES, INC.

SR-2508

HEAD AND NECK PROTOCOL

Objectives: To evaluate the following:

1. Does SR-2508 improve the local control rate of conventional radiotherapy in advanced head and neck cancers without unacceptable toxicity?

2. Does SR-2508 enhance the 2-5 year disease-free survival (cure) attained in this patient population?

3. Are any late effects of the combined treatment group upon normal tissues (skin/mucosa) different from radiotherapy alone?

Study Design: An open, randomized, parallel group design to assess the following treatments:

1. Radiotherapy alone -- 2.0 Gy per fraction, 5 fractions per week for a total of 66.0-74.0 Gy in 6.5-7.5 weeks.

2. Radiotherapy preceeded (30-60 min) by SR-2508, 2 gm/sq m, 3 times per week for 17 doses (34 gm/sq m).

ROBERTS LABORATORIES, INC.

SR-2508

HEAD AND NECK CARCINOMA STUDY

Scope: Approximately 300 patients in a multicenter program throughout Europe

Project Distribution:

France:	85-140
Italy:	125-150
Germany:	50- 58
Austria:	40- 50
United Kingdom:	40
	340-438

Projected Start: September 1986

ROBERTS LABORATORIES, INC.
SR-2508
ORGANIZATION OF CLINICAL PROGRAM

Institut Gustave Roussy
94805 Villejuif, France

- Dr. D. Chassagne, Principal Investigator
- Dr. E. P. Malaise, Preclinical Coordinator
- Dr. H. Sancho-Garnier, Biostatistician
- Dr. P. Henry-Amar, Biostatistician
- Dr. M. Bonnay, Drug Monitoring

Participating Centers	No. Patients Projected/ 12 Months
FRANCE	
- Institut Gustave Roussy	20-30
- Centre Claudius Regaud 11, rue Piquemil 31052 Toulouse Cedex	30-50
- Institut Jean Godinot 1, rue General Koenig - B.P. 171 51056 Reims Cedex	10-30
- Centre Alexis Vautrin 54511 Vandoeuvre Les Nancy Cedex	25-30
ITALY	
- U.S.L. Ventotto - Divisione Radioterapia Via Albertoni; 15 Bologna	50-60
- Institute di Radiologia Univ. Cattolica S. Cuore Largo A. Gemelli 8, Rome	30

ITALY (continued)	No. Patients Projected/ 12 Months
- Institute de Radiologia Univ. Viale Morgagni Careggi Firenze 50134 Firenze	15-20
- Universita Degli Study Di Torino 2a Cattedria di Radiologia Via Genova.3.C.A.P. 10126, Torino	30-40
GERMANY	
- Universitat Dusseldorf Klinik fur Strahlentherapie Moorenstrasse 5 D.4000 Dusseldorf 1	10
- Stadische Klinik - Radio-Onkologie D.4100 Duisburg	10
- Strahlentherapeutische Klinik Joseph Stelzmannstrasse 9 D.5000 Cologne 41	20-25
- Klinik fur Strahlentherapie Universitat Essen Essen	10-13
AUSTRIA	
- Strahlentherapie Universitats Klinik Anichstrasse 35 A-6020 Innsbruck	30
- Institut fur Radiotherapie und Radioonkologie Landeskrankenanstalten Salzburg Muller Hauptstrasse 48 A-5020 Salzburg	10-20

UNITED KINGDOM	No. Patients Projected/ 12 Months
- St. Lukes Hospital Warren Road Guildford Surrey Guz 5XX	20
- Radiotherapy Center Westminster Hospital London	20

ROBERTS LABORATORIES, INC.

SR-2508

T_3 BLADDER CARCINOMA STUDY

Study Site: National Cancer Institute, Cairo, Egypt. To enroll at least 150 patients with histologically proven T_3 bladder cancer who are able to undergo radical cystectomy.

Objectives: To evaluate the following:

1. Does SR-2508 improve the local control rate over radiotherapy alone without unnaceptable toxicity?

2. Does SR-2508 improve the one, two...five year tumor free survival (cure) in these patients?

3. Does SR-2508 have only acute or chronic effects over radiotherapy alone?

ROBERTS LABORATORIES, INC.

SR-2508

BLADDER PROTOCOL

Study Design: Open, randomized, parallel group design to evaluate the following treatments:

1. Cystectomy alone.
2. Cystectomy preceeded by concentration pre-operative radiotherapy; 5 daily fractions of 400 cGy each. Surgery 1-3 days following last dose.
3. Cystectomy preceeded by radiotherapy as above with SR-2508 (2.5-3 gm) given 30-60 min prior to radiotherapy.

TUMOR LEVELS

-- Tumor/plasma levels of SR-2508 will be determined in various head and neck and bladder patients.

-- SR-2508 metabolism will also be evaluated.

Ifosfamide
T83-1228 and
T84-0355
DFCC
July 1986

DANA FARBER CANCER INSTITUTE
IFOSFAMIDE FOR REFRACTORY SARCOMAS:

Patient Characteristics: 4/86
89 Patients: 11/83 - 11/85
Sex: M:44, F:45

	Median	Range
Age:	42	11-78
# Prior Regimens:	1	0-4
Drugs:	3	0-8

83 Evaluable for response

	#	#	95%CI
CR	6	7	
PR	15	18	
RR	21	25	16-36

		Median	Range
Duration of	CR	undef.	2-9+
	PR	4.3	1-13

Characteristics of responders
No significant differences

		#Pts	%CR	%RR
Age:	<18	6	0	0
	>18	77	8	27
Sex:	M	42	7	22
	F	41	7	28
Perf:	0	34	12	24
	1-2	49	4	26
Inf:	Bolus	60	7	25
		23	9	26

89 Patients entered
  1 Never received drug
88 Evaluable for toxicity
  2 Pulmonary edema D3
  1 Not a sarcoma
  1 Refused due to toxicity
  1 Hepatorenal failure
83 Evaluable for response

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Characteristics of responders

		#Pts	%CR	%RR
Cyclophos.:	No	32	13	35
	Yes	51	4	20
# Drugs:	0-2	28	11	21
	3-8	55	5	16
# Regimens:	0-1	45	9	29
	2-4	38	5	21

Ifosfamide
T83-1220 and
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IFF: 2G/sqM x 4D q 3 wks.
MESNA: Uroprotection
Pts. 1-64: 4 hour infusion/D
65-87: Cont. infusion over 4D to avoid neurotoxicity
Dose to 2.5 G/sqM/D for WBEC nadi- >1000

	# Pts	%CR	%RR
Leio	21	0	10
Osteo	13	15	15
MFH/Fib	9	11	70
Rhabdo	8	12	25
Neuro	5	0	20
Undif/Epit	5	40	60
Lipo	3	33	1/3
Syn	3	0	1/3
Chondro	2	0	2/2
Other	14*	0	0

*6 ES, 5 MM,. 1 angio, 1 giant cell

Total number of courses: 283
Toxicity: no deaths

	Courses	%Tox
WBC <1,000	213	23
Pltls: <20,000	207	2
HCO_3 <18	96	16
Creat >1.5	212	13
Confusion	242	7
Hematuria	229	3

Leiomyosarcoma

GYN	2 PR's/8
GI	0/6
STS	0/7
Total	2/21

AID FOR UNTREATED SARCOMAS

ADR:	30 mg/sqM/d x 3
IFF:	2.5g/sqM/d x 3
DTIC:	300 mg/sqM/ld x 3
MESNA:	2.5g/sqM/d x 4

42 Evaluable patients

	#	%	95%CI
CR	4	10	
PR	13	32	
RR	17	41	26-56

Elias, et al. Abst. #522

Ifosfamide
T83-1228 and
T84-0355
DFCC
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AID TOXICITY: 133 CYCLES
No deaths
WBC <500 33%
Plts. <50,000 14% DTIC (p=.03)
Moderate or Severe N/V 3%
Mucositis >GD 2: 2%
No CHF, 8 pts: 420-750 mg/sqM

AID: RESPONSES

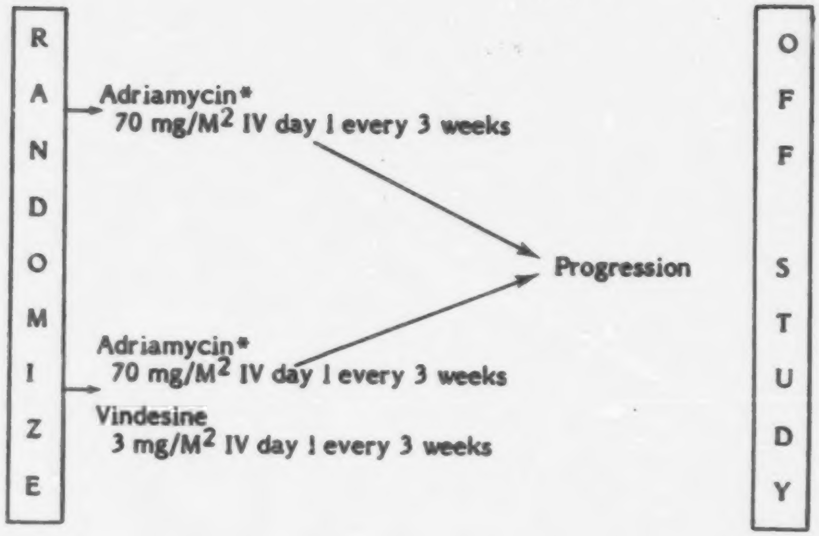
	#	%CR	%RR
LMS	13	0	31
MFH/fib	5	40	80
Neuro	5	0	40
ESS	4	50	75
Undif	4	0	25
Other	10	0	3/10

CONCLUSIONS
1. Iff is active in failed sarcomas.
2. MFH/Fib had a high response rate.
3. Single agent toxicity was acceptable.
4. IFF should be evaluated in a randomized trial in untreated patients.

SCHEMA - EST 2782

Stratification

Performance Status
Histopathologic Type
Site of Measurable
Disease


R A N D O M I Z E
Adriamycin*
70 mg/M2 IV day 1 every 3 weeks
Adriamycin*
70 mg/M2 IV day 1 every 3 weeks
Vindesine
3 mg/M2 IV day 1 every 3 weeks
Progression
OFF STUDY

*Suggested maximum total dose 550 mg/M^2

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EST 3377 - PHASE III

SCHEMA

Stratification

Performance status
Histopathologic type
Extent of disease

R A N D O M I Z E

No Prior Chemotherapy (Phase III)

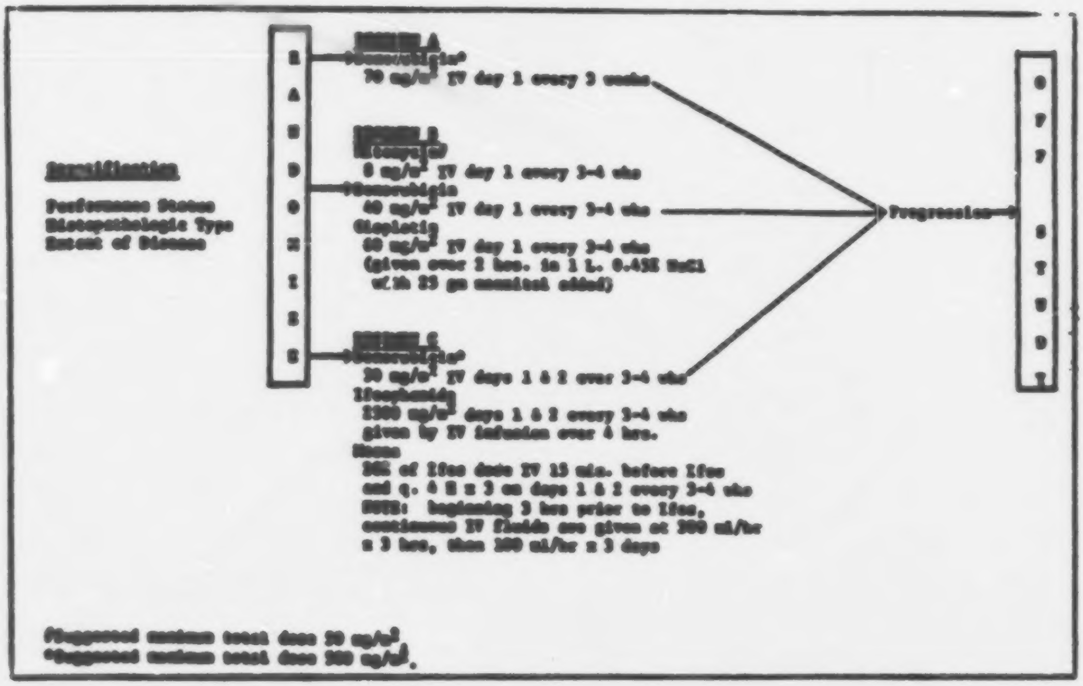
→Adriamycin 70 mg/M^2 IV d 1 & q 3 weeks

→Adriamycin 20 mg/M^2 IV d 1-3 and
and
15 mg/M^2 IV d 8 & q weekly

→Adriamycin 60 mg/M^2 IV d 1 & q 3 weeks
and
DTIC 250 mg/M^2 IV d 1-5 & d 1-5 q 3 weeks

EST 7-03

SCHEMA



Stratification

Performance Status
Histopathologic Type
Extent of Disease

R A N D O M I Z E

REGIMEN A
Doxorubicin*
70 mg/m^2 IV day 1 every 3 weeks

REGIMEN B
Mitomycin†
8 mg/m^2 IV day 1 every 3-4 wks
Doxorubicin
40 mg/m^2 IV day 1 every 3-4 wks
Cisplatin
60 mg/m^2 IV day 1 every 3-4 wks
(given over 2 hrs. in 1 L. 0.45% NaCl
with 25 gm mannitol added)

REGIMEN C
Doxorubicin*
30 mg/m^2 IV days 1 & 2 over 3-4 wks
Ifosfamide
1500 mg/m^2 days 1 & 2 every 3-4 wks
given by IV infusion over 4 hrs.
Mesna
20% of Ifos dose IV 15 min. before Ifos
and q. 4 H x 3 on days 1 & 2 every 3-4 wks
NOTE: beginning 3 hrs prior to Ifos,
continuous IV fluids are given at 300 ml/hr
x 3 hrs, then 100 ml/hr x 3 days

†Suggested maximum total dose 50 mg/m^2.
*Suggested maximum total dose 550 mg/m^2.

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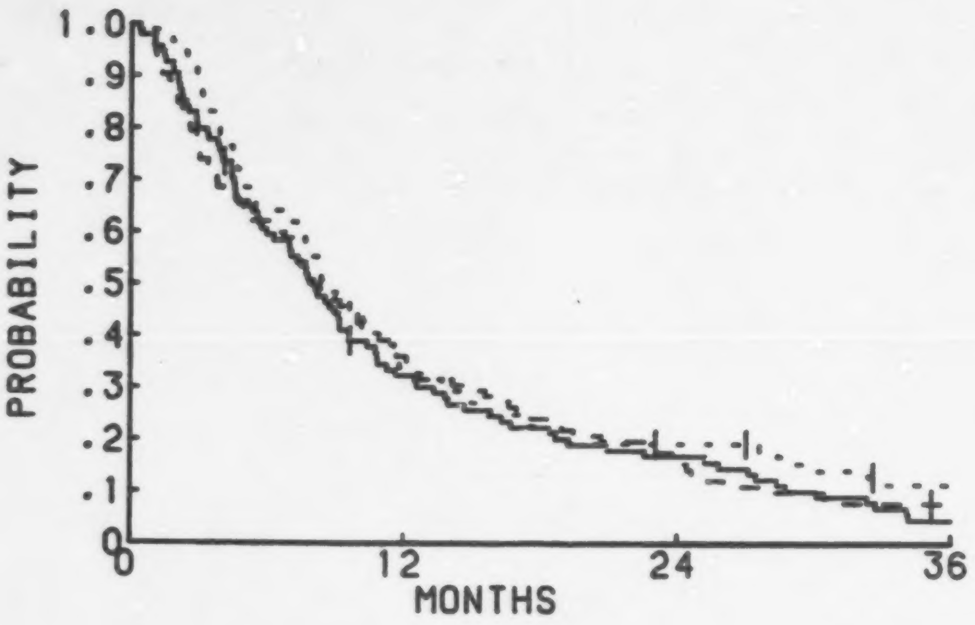
FIGURE 3.

SURVIVAL BY TREATMENT
SOFT TISSUE SARCOMA



TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
—— ADR Q3 WKS	3	91	94	8.0
- - - ADR WEEKLY	6	83	89	8.4
— — ADR + DTIC	1	91	92	8.0

RESPONSE BY SELECTED PATIENT CHARACTERISTICS

CHARACTERISTIC	A @ 3 WKS	A @ WK	A-DTIC	TOTAL	P-VAL.
OVERALL	18%	17%	30%	22%	
MALE	14%	17%	22%	18%	0.08
FEMALE	22%	17%	41%	27%	
AGE <50	19%	19%	19%	19%	0.45
AGE ≥50	18%	16%	36%	24%	
AMBULATORY	19%	19%	27%	22%	1.00
NONAMBULATORY	12%	7%	42%	22%	
LUNG METASTASES	12%	13%	28%	18%	0.05
NO LUNG METASTASES	26%	25%	34%	29%	
LEIOMYOSARCOMA	20% (7/35)	17% (6/36)	44% (12/27)	26% (25/98)	
MFH	29% (5/17)	6% (1/17)	25% (6/24)	21% (12/58)	

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CONCLUSIONS

1. WE HAVE CONFIRMED THAT A-DTIC HAS A HIGHER RESPONSE RATE THAN A ALONE. THE DIFFERENCE IS MAINLY IN THE PARTIAL RESPONSE RATES. A q 3 WKS AND A q WK APPEARED TO HAVE COMPARABLE RESPONSE RATES.

2. A-DTIC DID NOT APPEAR TO IMPROVE SURVIVAL.

3. A-DTIC WAS SIGNIFICANTLY MORE TOXIC, PARTICULARLY WITH REGARD TO NAUSEA/VOMITING. A q WK WAS LESS TOXIC, IN GENERAL.

4. A-DTIC REPRESENTS, AT BEST, A MODERATE ADVANCE. IT IS NOT CLEAR THAT THE INCREASED RESPONSE RATE, WITH NO IMPROVEMENT IN SURVIVAL, IS WORTH THE ADDITIONAL TOXICITY. ECOG HAS CHOSEN TO RETAIN A q 3 WKS AS ITS STANDARD REGIMEN.

5. THE RESPONSE RATE TO A-DTIC (44%) AMONG LEIOMYOSARCOMA PATIENTS WAS SIGNIFICANTLY HIGHER THAN TO A ALONE ($\leq$20%)

6. THE ON-STUDY DIAGNOSIS WAS CONFIRMED IN ONLY 2/3 PATIENTS. FOURTEEN PERCENT WERE REJECTED AS NOT SARCOMAS.

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TOXICITY FOR SOFT TISSUE SARCOMAS

NAUSEA/VOMITING	NONE	MILD	MOD.	SEV.	L-T	LETHAL
A @ 3 WKS	30%	19%	40%	11%		
A @ WK	33%	36%	26%	6%		
A-DTIC	10%	13%	48%	28%		
HEMATOLOGIC						
A @ 3 WKS	26%	22%	23%	26%	2%	
A @ WK	28%	36%	22%	12%	1%	
A-DTIC	22%	24%	24%	23%	7%	
CARDIAC						
A @ 3 WKS	90%	4%	2%	1%	1%	1%
A @ WK	94%	3%	1%	1%	-	-
A-DTIC	97%	-	-	2%	-	1%
WORST OVERALL						
A @ 3 WKS	5%	17%	39%	34%	3%	1%
A @ WK	3%	20%	56%	19%	1%	-
A-DTIC	-	2%	42%	48%	5%	2%
WORST (EXC.N/V)						
A @ 3 WKS	15%	22%	33%	26%	3%	1%
A @ WK	8%	25%	51%	16%	1%	-
A-DTIC	11%	12%	39%	30%	5%	2%

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RESPONSE - SOFT TISSUE

RESPONSE	A q 3 WKs	A q WK	A-DTIC	TOTAL
COMPLETE	5 (5%)	3 (3%)	6 (6%)	14 (5%)
	18%	17%	30%	22%
PARTIAL	12 (13%)	12 (14%)	22 (24%)	46 (17%)
NO CHANGE	10	6	11	27
PROGRESSION	67	68	53	188
TOTAL	94	88*	92	274
95% C.I. ON CR + PR RATE	(11%,27%)	(10%,26%)	(21%,41%)	(17%,27%)

COMPARISON	P-VALUE
OVERALL	0.06
A q 3 WK VS. A q WK	1.00
A q 3 WK VS. A-DTIC	0.06
A q WK VS. A-DTIC	0.04

* ONE LEIOMYOSARCOMA PATIENT WAS UNEVALUABLE FOR RESPONSE.

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PATIENT CHARACTERISTICS

CHARACTERISTICS	A @ 3 WKS	A @ WK	A-DTIC	TOTAL
MALE	52%	58%	55%	55%
AGE <30	11%	9%	13%	11%
30-49	29%	22%	21%	25%
50-69	51%	49%	54%	52%
>70	10%	14%	12%	12%
MEDIAN	56	58	58	57
P.S. 0-1	83%	82%	79%	82%
LEIOMYOSARCOMA	37%	42%	29%	36%
MFH	18%	19%	26%	21%
MALIGNANT SCHWANNOMA	4%	9%	10%	8%
SARCOMA, NOS	12%	3%	9%	8%
LIPOSARCOMA	6%	8%	8%	7%
FIBROSARCOMA	5%	6%	4%	5%
SYNOVIAL CELL SARCOMA	4%	6%	3%	4%
HEMANGIOPERICYTOMA	5%	3%	4%	4%
OTHER SARCOMA	5%	3%	4%	4%
RHABDOMYOSARCOMA	-	1%	1%	1%
ANGIOSARCOMA	2%	-	1%	1%

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COMPARISON OF ON-STUDY AND PATHOLOGY REVIEW DIAGNOSIS

FIBROSARCOMA	13	11	3	27
SPINDLE CELL SARCOMA	0	13	4	17
MALIGNANT SCHWANNOMA	12	3	0	15
MFH	39	5	7	51
LEIOMYOSARCOMA	83	6	10	99
SYNOVIAL CELL SARCOMA	8	3	1	12
RHABDOMYOSARCOMA	2	10	4	16
LIPOSARCOMA	17	4	1	22
ANGIOSARCOMA	2	3	3	8
HEMANGIOPERICYTOMA	7	3	0	10
OTHER SARCOMAS	16	11	12	39
TOTAL	199(63%)	72(23%)	45(14%)	316*

* FOUR PATIENTS NOT ENTERED AS SOFT TISSUE WERE CONFIRMED AS SOFT TISSUE AND ARE INCLUDED IN THE ANALYSIS (199+72+4 = 275). AN ADDITION 11 CLINICALLY EVALUABLE PATIENTS ARE NOT INCLUDED IN THIS TABLE DUE TO INSUFFICIENT MATERIAL (275+45+11 = 331). NOTE THAT 331-11-4 = 316.

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ADMINISTRATIVE INFORMATION - SOFT TISSUE

	A q 3 WKS	A q WK	A-DTIC	TOTAL
ENTERED	123	119	119	361
CANCELLED	4	4	2	10
INELIGIBLE	7	6	7	20
CLINICALLY EVALUABLE	112	109	110	331
NO PATHOLOGY REVIEW OR INSUFFICIENT MATERIAL	3	6	2	11
EXCLUDED BY PATH. REVIEW	15	14	16	45
CLINICALLY AND PATH. EVALUABLE	94	89	92	275

Ifosfamide
T83-1158
St. Jude
July 1986

RESPONSES OF SARCOMAS FOLLOWING IFOSFAMIDE

	NUMBER	CR	PR	MR	SD	PD
OSTEOSARCOMA	20	1	5	1	6	7
EWING'S SARCOMA	7	—	—	—	1	6
RHABDOMYOSARCOMA	5	—	2	—	—	3
OTHER SARCOMAS	9	1	3	2	2	1
TOTAL	41	2	10	3	9	17

CR + PR	12 OF 41 = .29
ANY RESPONSE	24 OF 41 = .59

SJCRH 7/86

RELATIONSHIP OF PRIOR ALKYLATING AGENT TREATMENT TO RESPONSE WITH IFOSFAMIDE

PRIOR	TOTAL	CR	PR	MR	SD	NR
CYCLO	22	—	6	2	3	11
DDP	9	1	2	—	4	2
DDP + CYCLO	6	—	2	1	1	2
NEITHER	4	1	—	—	1	2
	41	2	10	3	9	17

- IFOSFAMIDE HAS NOT BEEN TESTED IN PATIENTS WITH BRAIN TUMORS
- IFOSFAMIDE HAS NOT DEMONSTRATED ACTIVITY IN PEDIATRIC PATIENTS WITH HEPATIC TUMORS, CARCINOMAS

OTHER PEDIATRIC TUMORS RESPONSIVE TO IFOSFAMIDE

- NEUROBLASTOMA
- WILMS' TUMOR
- PRIMITIVE NEUROECTODERMAL TUMOR
- RETINOBLASTOMA
- DYSGERMINOMA
- EMBRYONAL CARCINOMA
- NON-HODGKIN'S LYMPHOMA

Ifosfamide
T83-1158
St. Jude
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WHAT IS NEEDED?

- DETERMINE MAXIMUM TOLERATED DOSAGE FOR PEDIATRIC SUBJECTS
- TEST ALTERNATIVE SCHEDULES
- INITIATE COMBINATION TREATMENTS USING IFOSFAMIDE FOR SARCOMAS, I.E., OSTEOSARCOMA
- METHODS TO PREDICT OR PREVENT NEUROTOXICITY

POSSIBLE APPLICATIONS OF IFOSFAMIDE IN PEDIATRIC MALIGNANCIES

- FURTHER INVESTIGATION AS A SINGLE AGENT IN SOFT TISSUE SARCOMAS, GERM CELL TUMORS, LYMPHOMAS AND RARE TUMORS
- USE IN COMBINATION FOR:
  - SARCOMAS, ESPECIALLY OSTEOSARCOMA
  - NEUROBLASTOMA
  - WILMS' TUMOR

Ifosfamide
T85-0225
NCI
July 1986

IFOSFAMIDE - MESNA - ETOPOSIDE

TREATMENT SCHEMA

IFOSFAMIDE	1800 mg/square meter/day x 5 days
MESNA	2880 mg/square meter/day x 5 days
ETOPOSIDE	100 mg/square meter/day x 5 days

CYCLES ARE REPEATED EVERY THREE WEEKS

A TOTAL OF TWELVE CYCLES ARE GIVEN

PATIENT CHARACTERISTICS

MEDIAN AGE	19 YEARS (RANGE: 1 to 42 YEARS)
GENDER	46 FEMALES: 70 MALES
MEDIAN # OF PREVIOUS REGIMEN	1 (RANGE 1 to 7)
RESISTANT TO STANDARD THERAPY	62 PERCENT
PREVIOUS CYCLOPHOSPHAMIDE	97 PERCENT
MEDIAN PREVIOUS ADRIAMYCIN	395 MG/SQUARE METER

DIAGNOSES

EWING'S SARCOMA	30
RHABDOMYOSARCOMA	17
PERIPHERAL NEUROEPITHELIOMA	11
OSTEOSARCOMA	15
SPINDLE CELL SARCOMA	23
PRIMITIVE SARCOMA	4
NEUROBLASTOMA	1
OTHER	15

IFOSFAMIDE - MESNA - ETOPOSIDE

RESPONSE TO CHEMOTHERAPY

TOTAL NUMBER OF PRESENTLY EVALUABLE PATIENTS	67
COMPLETE RESPONSE	4
PARTIAL RESPONSE	34
STABLE DISEASE	10
PROGRESSIVE DISEASE	19

IFOSFAMIDE - MESNA - ETOPOSIDE

RESPONSE TO CHEMOTHERAPY

EWING'S SARCOMA	13/14
RHABDOMYOSARCOMA	8/12 (3 CR'S, 5 PR
PERIPHERAL NEUROEPITHELIOMA	4/8
OSTEOSARCOMA	4/9
NEUROBLASTOMA	0/1
PRIMITIVE SARCOMA	1/3
SPINDLE CELL SARCOMA	5/12
OTHER TUMORS	3/8 (1 CR, 2 PR'S)

IFOSFAMIDE - MESNA - ETOPOSIDE

TOXICITY

ORGAN SYSTEM TOXICITY	PERCENT OF COURSES WITH MODERATE OR SEVERE TOXICITY (GRADE III OR IV)
NEUTROPENIA	97 %
THROMBOCYTOPENIA	32 %
HEPATOTOXICITY	4 %
NEPHROTOXICITY	<1 %
MUCOSITIS	1 %
NEUROLOGIC TOXICITY	2 %
NAUSEA OR VOMITING	13 %
HEMORRHAGIC CYSTITIS	<1 %

IFOSFAMIDE - MESNA - ETOPOSIDE

INFECTIOUS COMPLICATIONS

SEVERE NEUTROPENIA (ANC <500)	94 %
FEVER	33 %
SEPSIS	7 %
DEATH DUE TO INFECTION	0.3 % (1 PATIENT)

CONCLUSIONS

1. IFOSFAMIDE - MESNA - ETOPOSIDE IS AN EFFECTIVE REGIMEN IN THE TREATMENT OF RECURRENT CHILDHOOD TUMORS AND SARCOMAS.
2. MESNA IS AN EFFECTIVE UROPROTECTOR.
3. THIS COMBINATION SHOULD BE EVALUATED IN NEWLY DIAGNOSED, HIGH RISK SARCOMAS NOW.

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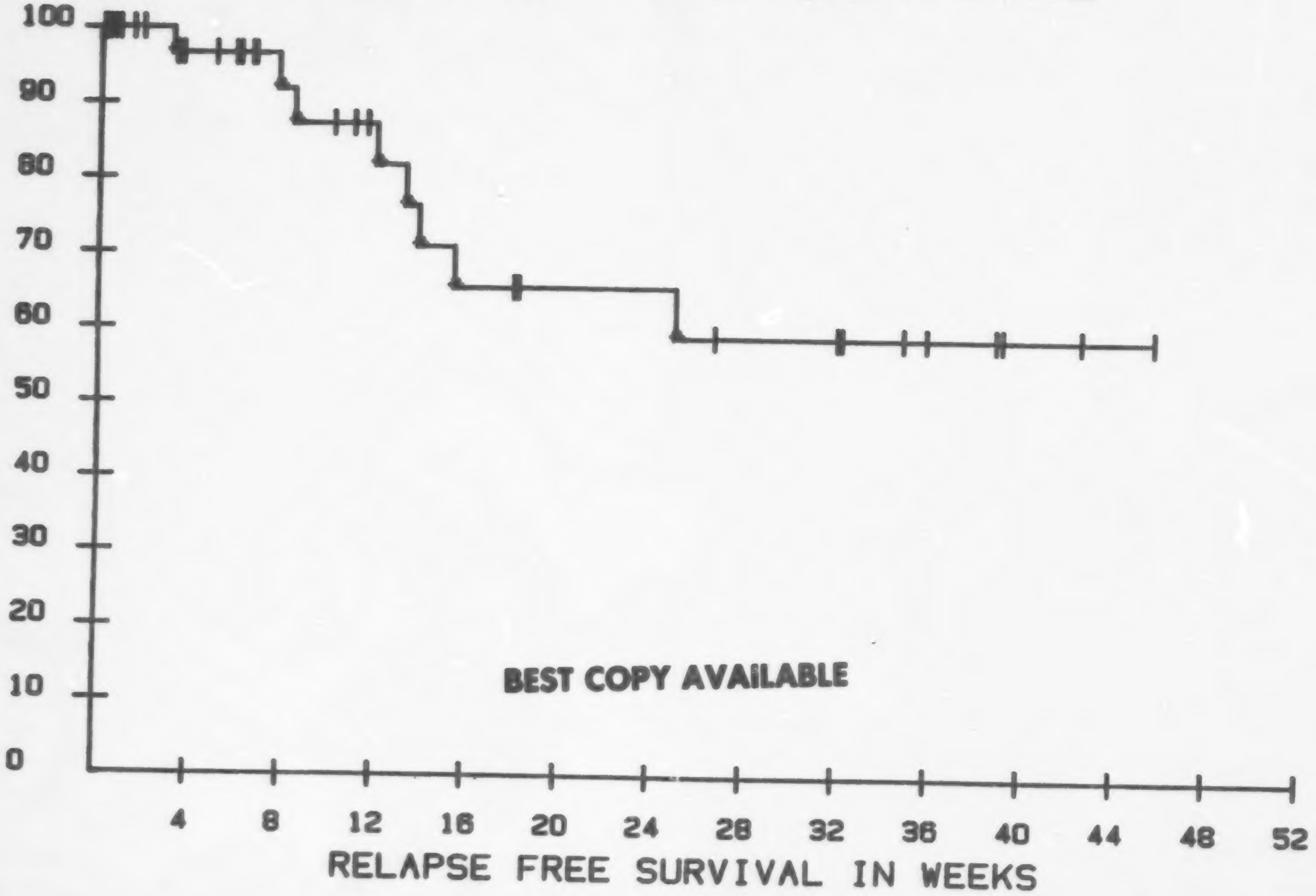
IFOSFAMIDE - MESNA - ETOPOSIDE RELAPSE FREE SURVIVAL



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IDENTIFICATION OF CHLOROACETALDEHYDE IN PATIENTS RECEIVING IFOSFAMIDE; POTENTIAL RELATIONSHIP TO NEUROTOXICITY

MARSHALL GOREN, M.D.
ST. JUDE CHILDREN'S RESEARCH HOSPITAL
MEMPHIS, TENNESSEE

DIFFERENCES IN CLINICAL TOXICITY

	IFOSFAMIDE	CYCLOPHOSPHAMIDE
BLADDER TOXICITY	+	+
HEMATOPOIETIC TOXICITY	+	+
NEPHROTOXICITY	+	0
NEUROTOXICITY	+	0

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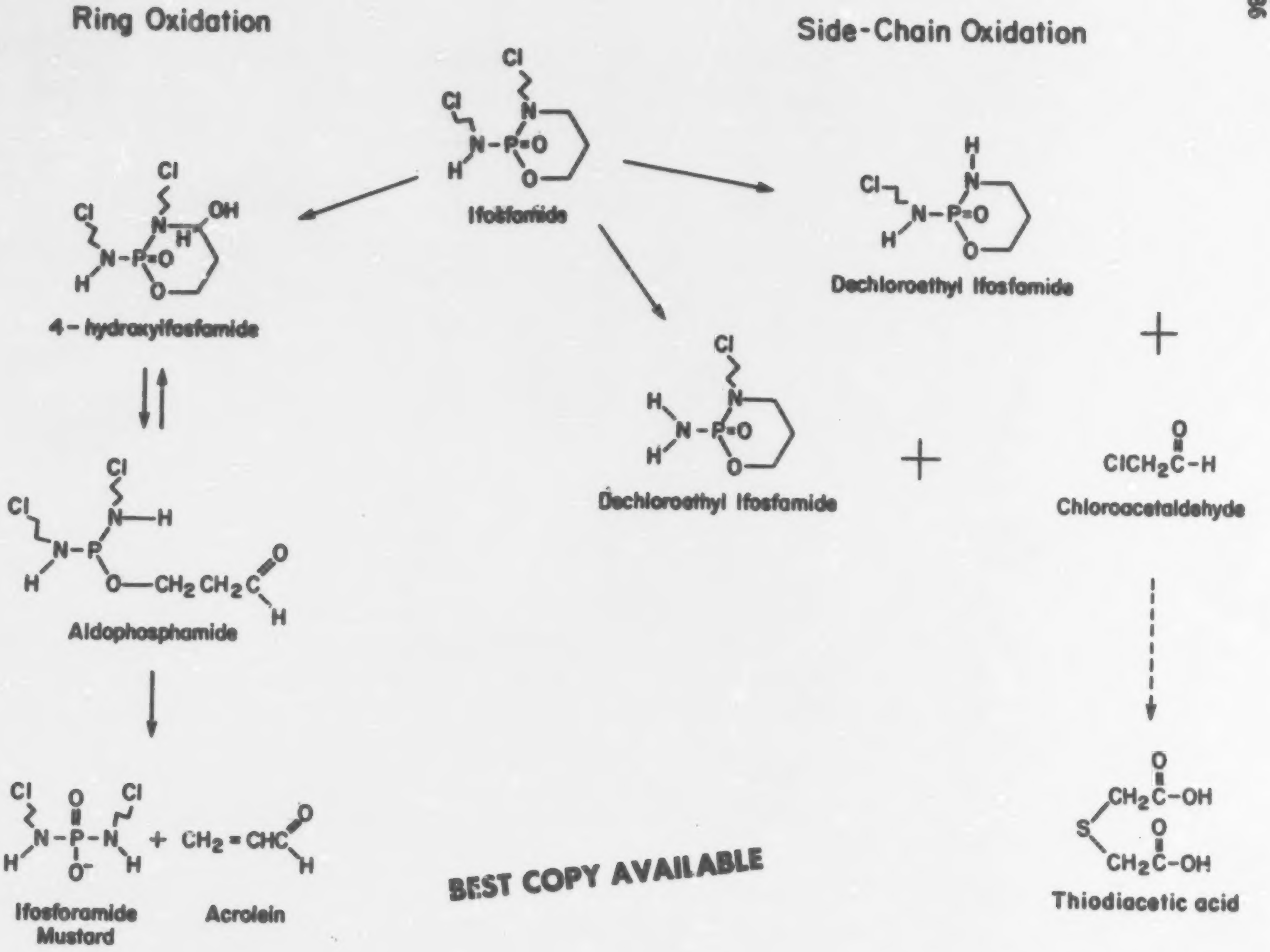
Ring Oxidation
Side-Chain Oxidation
Ifosfamide
4-hydroxyifosfamide
Aldophosphamide
Ifosforamide Mustard
Acrolein
Dechloroethyl Ifosfamide
Dechloroethyl Ifosfamide
Chloroacetaldehyde
Thiodiacetic acid

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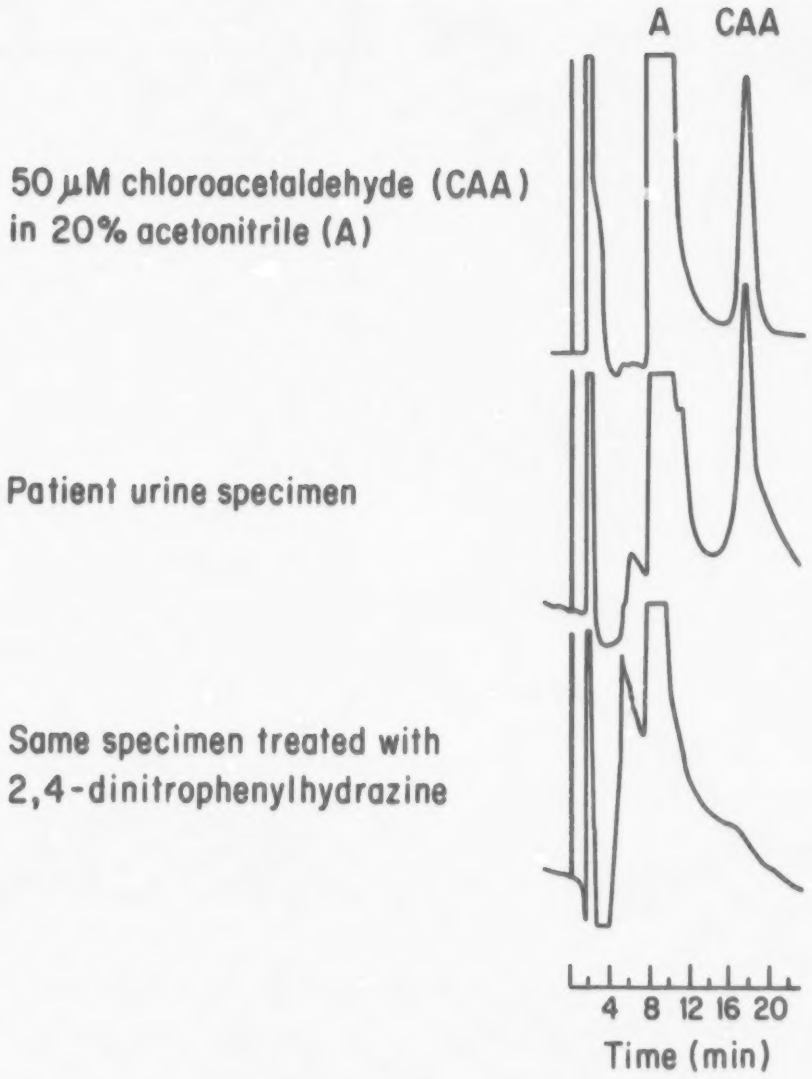

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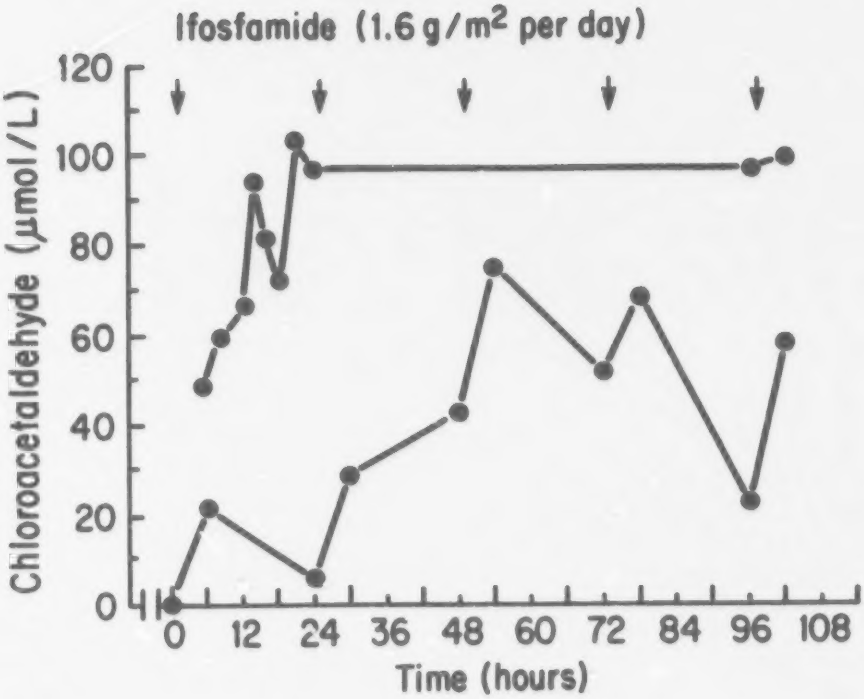

CONCLUSION

- CHLOROACETALDEHYDE WAS IDENTIFIED IN BLOOD, CSF AND URINE.

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VP-16 Plus Ifosfamide Plus Cisplatin as Salvage Therapy in Refractory Germ Cell Cancer

By Patrick J. Loehrer, Sr, Lawrence H. Einhorn, and Stephen D. Williams

Forty-eight evaluable male patients with germ cell tumors (GCT) failing to be cured with first-line therapy were treated with VP-16 (75 mg/m^2), ifosfamide (1.2 g/m^2), and cisplatin (20 mg/m^2) (VIP), all given daily for 5 consecutive days every 3 weeks. All patients either achieved an unresectable partial remission as their best response to induction chemotherapy (Group A), relapsed from complete remission (CR) ≤ 2 months after induction therapy (Group B), or had received cisplatin plus VP-16 as previous salvage therapy (Group C). Nine (19%) had extragonadal GCT, and 37 (77%) had advanced disease. Twenty-three (48%) of the patients had ≥ 2 prior treatment regimens. Sixteen of 48 (33%) achieved CR with VIP treatment alone or following surgical excision of residual disease. Six of 22 (27%), three of seven (43%), and seven of 19 (37%) patients from groups A, B, and C, respectively, attained a CR. The median survival time of all patients was 7 months (range 0 to 28+) with seven patients remaining continuously free of disease (four patients > 1 year). Myelosuppression was significant with a median WBC nadir of 900/mm^2 and platelet nadir of 24,000/mm^2. Fourteen (26%) had granulocytopenic fever, and renal insufficiency developed in 15%. VIP combination chemotherapy demonstrates activity in this highly unfavorable population of patients with germ cell tumors. The actual contribution of ifosfamide in this regimen is unclear, but these results compare favorably to our experience with similar patients treated with cisplatin plus VP-16 alone. Further studies with VIP as initial salvage therapy for patients with GCT are planned.

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DRAMATIC STRIDES have been made in the treatment of metastatic testicular cancer. Currently, cisplatin-based combination regimens can be expected to produce complete remissions (CRs) in 70% to 80% of treated patients.[1,2] For the remainder of these patients and for those who relapse from CR, salvage chemotherapy remains the only alternative for cure. If they are not initially cured with cisplatin, vinblastine, and bleomycin, further treatment with these agents might produce second remissions, but these are transient in nature. VP-16, an epipodophyllotoxin, is one of the few agents that has been found to have single-agent activity in cisplatin-refractory patients.[3-5] Indeed, cisplatin plus VP-16 have produced together or as part of combination regimens durable CRs in 25% of previously treated patients.[3,6-10]

Ifosfamide, an oxazaphosphorine, is an analogue of cyclophosphamide that differs from its parent compound in that myelosuppression is less common and hemorrhagic cystitis is the dose-limiting side effect.[11,12] With the development of effective uroepithelial protective agents (eg, *N*-acetylcysteine [NAC], mesna), the therapeutic index has changed.[13,14] Several European investigators have reported single-agent activity for ifosfamide in refractory testicular cancer. In 18 patients with advanced testicular cancer (11 previously treated), 15 patients responded including two complete and 13 partial remissions (PRs). The median remission time was 5.4 months, and the median survival time of the responders was 11 months.[15] Similar responses have been reported by others[16,17]; interpretation of these studies has been difficult since prior dosages and scheduling of chemotherapy as well as previous response were not readily accessible. Additionally, it was unclear as to how many patients were truly refractory to induction chemotherapy (ie, progressing within 3 weeks of the previous course of therapy) *v* late relapses from CRs.

From the Department of Medicine, Indiana University Hospital, Indianapolis.

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P.J.L. is an American Cancer Society Junior Faculty Fellow.

L.H.E. is an American Cancer Society Clinical Professor of Oncology.

Address reprint requests to Patrick J. Loehrer, Sr, MD, Department of Medicine, Indiana University Hospital, Indianapolis, IN 46224.

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Table 1. Modified M.D. Anderson Staging System

- A. Minimal pulmonary disease
- B. Advanced pulmonary disease
- C. Minimal abdominal with or without pulmonary disease
- D. Advanced abdominal with or without pulmonary disease
- E. Serum marker only
- F. CNS, bone, extra-abdominal nodal, etc

NOTE: Minimal is defined as stage A, C, or E; advanced is defined as stage B, D, or F.

Based on these results, we embarked on a phase II trial investigating the single-agent activity of ifosfamide in patients with germ cell tumors refractory to cisplatin.[18] In 30 patients with advanced disease who were treated with ifosfamide (2 g/m²/d) for 5 consecutive days every 3 weeks, there were six PRs and one CR (overall response rate 23%). The median duration of response was 3½ months, with a median survival time for all patients of 3½ months. Unfortunately, as was true with single-agent VP-16, ifosfamide failed to produce durable CR in any of these treated patients. Although this amount of activity was not as high as reported by other investigators, it was impressive to use since in our experience, VP-16 was the only other agent to demonstrate activity in patients truly refractory to cisplatin.

As a result of this single-agent activity and known preclinical synergy of oxazaphosphorines plus cisplatin,[19,20] we embarked on a trial using cisplatin, ifosfamide, plus VP-16 (VIP) as salvage therapy for patients with refractory germ cell tumors.

MATERIALS AND METHODS

From February 1983 through October 1984, 50 previously treated patients with advanced germ cell tumors were seen at Indiana University and treated with chemotherapy. All but one of these patients had previously received cisplatin-vinblastine-bleomycin combination chemotherapy and failed to be cured. One patient had received vincristine in place of vinblastine in a fashion previously described by Wettlaufer et al.[21] Forty-one patients had primary testicular cancer whereas nine patients had primary extragonadal germ cell tumors (retroperitoneum, two; mediastinum, seven). Patients entered into the study had either achieved an unresectable PR as a best response to initial induction chemotherapy (group A), a CR but relapse within 2 months of completing induction chemotherapy (group B), or also had received cisplatin plus VP-16 as salvage therapy but subsequently relapsed (group C). Two patients were ineligible for the protocol (one patient with a falsely elevated marker and one patient without measurable disease) leaving 48 fully evaluable patients. Through a modification of the M.D. Anderson staging system (Table 1), 11 patients were classified with minimal and 37 patients were classified with advanced disease. Patient characteristics are described in Table 2.

The VIP treatment regimen consisted of VP-16 (75 mg/m²), ifosfamide (1.2 g/m²), and cisplatin (20 mg/m²), all given daily for 5 consecutive days. In addition, the uroepithelial protective agent NAC was administered at a dosage of 2.0 g (four 500-mg capsules supplied by Mead-Johnson Pharmaceutical Co, Evansville, Ind) orally every six hours starting within one hour before ifosfamide and continued until 36 hours after the last dose of

Table 2. Patient Characteristics

	Group A (N = 22)	Group B (N = 7)	Group C (N = 19)	Total (%)
Histology				
Embryonal carcinoma	6	1	6	13 (27)
Teratocarcinoma	6	3	7	16 (33)
Choriocarcinoma	2	0	1	3 (6)
Seminoma	1	1	0	2 (4)
Combination of above	7	2	5	14 (29)
Site of primary				
Testis	18	5	16	39 (81)
Mediastinum	3	2	2	7 (15)
Retroperitoneum	1	0	1	2 (4)
Stage (defined in Table 1)				
Minimal	2	2	7	11 (23)
Advanced	20	5	12	37 (77)
Previous treatment				
2 previous regimens	2*	1	11	14 (29)
≥ 3 previous regimens	1*	0	8	9 (19)
Prior cyclophosphamide	2	0	4	6 (12.5)
Previous radiotherapy (excluding brain)	1	0	2	3 (6)

*Not containing VP-16.

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ifosfamide. Patients with prior abdominal, pelvic, or chest radiotherapy received a 25% reduction in both ifosfamide and VP-16. A urinalysis was repeated daily, and if greater than ten red blood cells per high-powered field were noted, ifosfamide was withheld until the hematuria resolved while the NAC administration was increased to every four hours. If hematuria recurred upon treatment with ifosfamide, then subsequent ifosfamide dosages were decreased by 25%.

Courses were repeated every 21 days for four cycles. Dosage modifications included a 25% reduction in both ifosfamide and VP-16 for patients in whom granulocytopenic fever or thrombocytopenic bleeding developed. Patients who demonstrated an increase in the serum creatinine level ≥ 2 mg/dL had their chemotherapy withheld until the creatinine level stabilized, with subsequent ifosfamide dosages reduced by 25%.

Pretreatment evaluation consisted of physical examination, urinalysis, complete blood count, sequential multiple analyzer (SMA-12), and a determination of the levels of electrolytes, creatinine, B-subunit human chorionic gonadotropin (BHCG) and α-fetoprotein (αFP). Chest roentgenogram and computerized tomography of the abdomen or chest were performed when appropriate for measurements. Chest roentgenogram and all blood tests including serum markers were repeated every 3 weeks. Computerized tomography was repeated at 9 weeks or as indicated to document remission. When feasible, resection of residual disease occurred 6 to 8 weeks after the fourth course of therapy. If resected disease revealed carcinoma, two additional courses of VIP were administered.

CR was defined as no evidence of disease by physical examination, roentgenographic studies, and serum markers lasting at least 1 month. A PR was defined as a $\geq 50\%$ reduction in the extent of disease using the aforementioned criteria as measured by the sum of the products of perpendicular diameters of bidimensionally measurable lesions and including a 90% or greater reduction in the pretreatment level of BHCG or αFP. Response duration was measured from the date of documented response or surgical excision of residual disease until progression. Survival time was measured from the first day of treatment until death. All patients entered receiving at least one day of therapy were considered eligible for response and survival.

RESULTS

Clinical Response: All patients

Thirteen patients achieved a CR, including four patients who underwent resection of residual disease revealing necrosis or fibrosis. Additionally, one patient underwent resection of teratoma, and two patients underwent resection of carcinoma following VIP therapy (total CR + surgery with no evidence of disease [NED], 16 of 48 [33%]) (95% confidence limits are 20% and 47%). Twenty of the 48 (42%) patients achieved a PR, whereas 12 (25%) failed to respond. The median duration of remission for complete responders was 9 months (range, 2 to 26+), compared with a median duration of remission of only 2 months for partial responders. The median survival time for all evaluable patients was 7 months (range, 0 to 28+ months).

Four out of 11 (36%) patients with minimal and 12 of 37 (32%) with advanced disease achieved a favorable clinical response to VIP. Three of the six patients who had received previous treatment with regimens containing cyclophosphamide attained a CR (two CR, one NED carcinoma). Two of these latter three patients had not achieved a previous CR.

Fourteen of 39 (36%) patients with primary testicular carcinoma favorably responded, compared with two of the nine patients with primary extragonadal germ cell tumors (both with primary retroperitoneal tumors). In reference to histology, favorable responses in patients occurred in 100% (2/2) of seminoma, 31% (4/13) of embryonal carcinoma, 33% (1/3) of choriocarcinoma, 19% (3/16) of teratocarcinoma, and 43% (6/14) with other mixed or unclassifiable germ cell neoplasms. Results of each of the subgroups are detailed individually.

Clinical Response: Subgroups

In group A, six of 22 (27%) patients achieved a CR (95% confidence limits are 9% and 46%). Details of these patients are outlined in Table 3. Three of these six patients remain free of disease 10, 23, and 26 months from the start of therapy. An additional patient with extensive prior whole-brain radiotherapy (both external and interstitial) died of a pulmonary embolus 9 months from the initiation of chemotherapy. No viable tumor was found at autopsy.

Three of the seven patients from group B attained a second CR with VIP treatment lasting 3, 5, and 10+ months (Table 3).

In group C, seven of 19 (37%) patients achieved a CR (95% confidence limits are 17% and 58%). The median duration of remission for this group was 9 months (range, 5 to 21+ months). Three patients remain alive and in remission 9, 17, and 25 months, respectively (Table 3).

Toxicity

Nausea and vomiting occurred in virtually all patients, but was severe despite antiemetic therapy in only ten patients. Myelosuppression was severe, with 75% of the patients having a WBC nadir $\leq 1{,}000/\text{mm}^2$ and 33% $\leq 500/\text{mm}^2$. Simi-

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larly, thrombocytopenia was also severe, with 66% of the patients having a platelet count nadir $\leq 50{,}000/mm^2$ and 26% $\leq 20{,}000/mm^2$. The median WBC and platelet nadirs were $900/mm^2$ and $24{,}000/mm^2$, respectively. Granulocytopenic fever including three episodes of documented sepsis developed in 14 patients (26%).

Nephrotoxicity (serum creatinine level ≥ 2 mg/100 mL) occurred in seven (15%) patients including three patients with creatinine levels ≥ 6 mg/100 mL. None of these patients required dialysis. Macroscopic hematuria developed in seven (15%) patients. One additional patient complained of dysuria without hematuria. Other side effects included headaches in two patients, and in one patient each, congestive heart failure and hypokalemia occurred. Only four otherwise-responding patients received less than two courses of therapy because of gastrointestinal or urological side effects. There were no drug-related deaths.

DISCUSSION

Patients failing to be cured with induction chemotherapy generally have a bleak prognosis. Platinum–VP-16 combination regimens have nonetheless produced durable CRs in approximately 25% of patients. For the remainder of these patients, unique forms of therapy are obviously indicated.

Predictive prognostic factors for salvage therapy in testicular cancer are difficult to elucidate because of small numbers of such patients. In our experience, however, the failure to achieve CR or relapsing quickly (≤ 2 months) from a CR has been associated with few long-term survivors.[22] In support of this, Bosl and his colleagues reported only five PRs and no CRs using cisplatin plus etoposide in 28 patients with previously unresectable PR to induction chemotherapy.[10] In contrast, Hainsworth et al reported seven complete responses in 23 (30%) patients using these same agents in patients with previously unresectable PR.[9] Four of these seven patients who had been treated at Indiana University, however, did demonstrate normalization of serum marker levels with induction chemotherapy but were left with persistent roentgenographic abnormalities before they later demonstrated progressive disease. In this present series, 16 of 22 patients defined as unresectable PRs had previously failed to normalize serum marker levels with induction chemotherapy.

Thus, the term "unresectable PR" has a variable definition. Prospectively, patients who have roentgenographic plus concurrent serologic abnormalities clearly meet this definition. For those patients, however, with residual radiographic disease and normal marker levels following induction chemotherapy, the term unresectable PR is not always appropriate. Although some patients will later relapse with progressive cancer manifested by serum markers and/or roentgenographically, other patients may continue to show regression (implying necrotic tumor) or stable disease (implying teratoma). In the group of patients with roentgenographic abnormalities and normal marker levels, only when progressive disease develops can the patient be correctly (but retrospectively) known to have residual cancer. Whether these subgroups of unresectable PR have prognostic differences remains uncertain. Thus, because of uncertainties in the historical data, it remains indeterminate from this series as to whether or not ifosfamide adds to the known effectiveness of cisplatin plus etoposide as salvage therapy in patients with unresectable PR, but these results favorably compare with our previous experience with such patients.

For patients who have previously received VP-16 plus cisplatin salvage chemotherapy, however, the potential contribution of ifosfamide to cisplatin–VP-16 combination becomes easier to document. A patient who has failed to be cured with an induction or salvage regimen given in appropriate dosages and in a timely fashion may occasionally respond but will never in our experience achieve a durable CR when using the same regimen(s). Thus, in group C in which all the patients had previously received VP-16 and cisplatin any patient who has a durable CR should demonstrate the therapeutic contribution of ifosfamide.

Indeed, in this latter group, seven of 19 (37%) patients achieved a CR, and three (16%) remain free of disease at 9+, 17+, and 25+ months after the initiation of chemotherapy. Observation is needed, but these results appear quite encouraging as third-line therapy.

Although there were no drug-related deaths in this series, toxicity (particularly myelosuppression) was formidable. Nephrotoxicity was also

Table 3. Clinical Data on Complete Responders to VIP

Patient	Pathology	Primary Site	Prior Therapy	Response to Prior Therapy	Metastatic Sites	Dosage Extent*	Response to VIP	Remission Duration (mo)	Survival (mo)
Group A									
1	C	RP	PVB × 3	PR†	lung, liver, BHCG, RPLN	D	CR	28 +	31 +
2	S/T	testis	POB × 4	PR	liver, lung, RPLN	D	CR	2	7
3	EC	testis	PVB × 3	PR†	lung, RPLN	D	CR	9 +	11 +
4	EC/YS/C	testis	PVB × 4	PR†	brain, lung, RPLN, BHCG, αFP	D	CR (NED, necrosis)	6	9‡
5	S/T	testis	PVB × 1	NR†	lung, BHCG	D	CR	20 +	24 +
6	S	testis	XRT (ID)	PR	RPLN	B	CR	14	22 +
			PVB × 5	PR					
			cyclophosphamide	PR					
			PVB	NR					
			XRT (SD)	PR					
Group B									
7	T/EC	testis	PVB × 4	CR	lung, mediastinum	D	CR (NED, necrosis)	5	7
8	EC	testis	PVB × 6 (NED CA)	CR	lung	B	CR	3	8
9	S	testis	XRT (ID) PVB × 4	CR	HCG	E	CR	10 +	13 +
Group C									
10	T/EC	testis	PVB × 4	PR	lung, liver, αFP	D	CR (NED-T)	8	12
			BEP × 1	PR					
			surgery BEP × 4	PR					
11	S	testis	XRT (ID/SD)	CR	bone, RPLN	F	CR	12	17 +
			PVB × 4	CR	adrenal, αFP				
			BEP × 4	CR	BHCG, thyroid				
12	EC	testis	PVB × 4	PR	lung, RPLN, αFP	C	CR	14 +	17 +
			BEP × 2	CR					

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13	EC	testis	XRT (ID)	NR	RPLN, aFP	C	CR	6+	9+
			ACT/V/B × 5	NR					
			V × 7	NR					
			DOX × 7	NR					
			CMFO × 2	NR					
			BCG P × 3	PR					
			surgery (lung)	T					
			DOX	NR					
			surgery	CR					
			PE	CR					
14	EC/T	testis	PVB	CR	RPLN, lung	D	CR (NED-T)	5	24§
			PE	PR	aFP				
15	undifferentiated/T	RP	PVB/A × 8	PR	lung	E	CR	9	17‖
			PE × 4	PR	HCG				
16	C/EC/T	testis	PVB/PVA × 6	CR¶	RPLN, HCG	C	CR (NED, carcinoma)	21+	25+
			PVB × 4	CR					
			PE × 4	CR					

Abbreviations: PVB, cisplatin, vinblastine, bleomycin; POB, cisplatin, vincristine, bleomycin; BEP, bleomycin, etoposide, cisplatin; PVA, cisplatin, vinblastine, doxorubicin; PE, cisplatin, etoposide (VP-16); B, bleomycin; V, vinblastine; ACT, actinomycin D; DOX, doxorubicin; BCG, bacillus Calmette-Guerin; CMFO, cyclophosphamide/methotrexate/5-fluorouracil/vincristine; P, cisplatin; XRT, radiation therapy; (ID), infradiaphragmatic; (SD), supradiaphragmatic; RPLN, retroperitoneal lymph node; RP, retroperitoneum; NR, no response; S, seminoma; YS, yolk sac carcinoma; EC, embryonal carcinoma; T, teratoma; C, choriocarcinoma; CA, carcinoma.

*See Table 1.

†Markers did not normalize with therapy.

‡Patient died of pulmonary embolus with no viable tumor at autopsy.

§Received only 2 days of ifosfamide secondary to hematuria.

‖Relapsed with brain metastases treated only with XRT.

¶Initial remission to PVB/A lasted 66 months. Subsequent remission durations with PVB and PE were 6 months and 14 months, respectively.

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seen in several patients. As one might expect when using drugs that demonstrated preclinical therapeutic synergy, toxicities from combination agents might also be additive if not synergistic. The degree of myelosuppression as well as nephrotoxicities in this series was greater than what is usually seen with cisplatin plus VP-16 therapy without ifosfamide.

Another question is whether similar high-dose chemotherapy without ifosfamide with equivalent myelotoxicity could achieve comparable results to VIP chemotherapy. High-dose chemotherapy (usually using VP-16) administered with autologous marrow transplantation is a logical application of this concept. Despite several CRs achieved with such approaches in testicular cancer (Table 4), responses are brief, and long-term survival has not been observed.

High doses of cisplatin would appear to be another practical approach since a dose-response relationshp was observed by the Southwest Oncology Group, which compared 75 mg/m² to 120 mg/m² in first-line therapy.[27] Ozols and his colleagues extended this concept by using cisplatin, 40 mg/m² daily × 5 with hypertonic saline with encouraging results as first-line therapy in patients with advanced disease.[28] Whether these high dosages of cisplatin can overcome resistance in patients previously treated with conventional cisplatin dosages is uncertain. Using high doses of cisplatin (40 mg/m² daily × 5) with VP-16 (100 g/m² daily × 5), Trump and Hortvet recently reported their experience with 12 such patients.[29] Three of the 12 (25%) patients had attained previous short-term CR (3, 3, 5 months), with the nine remaining patients having PR as their best responses to PVB. The median nadir WBC and platelet count were 1,200/mm² and 18,500/mm², respectively. Therapy was associated with granulocytopenic fever in 14 of 33 cycles. A clinically evident hearing loss developed in five patients, although clinically significant renal insufficiency did not occur. Four patients achieved CR to this salvage regimen (two, chemotherapy only; two, NED carcinoma). One additional patient had radiation therapy to residual abdominal seminoma. Only one patient, however, remained in CR 24 months after therapy, with the other complete responders relapsing between 2 and 4 months. On the basis of these studies, it would appear that neither high-dose VP-16 nor high-dose cisplatin-based combination chemotherapy salvage regimens have a significant advantage over conventional dosage therapy to date.

Since the development of effective first-line regimens in testicular cancer with cisplatin, the effects of cyclophosphamide in second-line therapy has been largely unknown. In our experience, using a variety of different regimens with a cyclophosphamide combination regimen has rarely produced favorable responses. There remains sparse clinical data, however, that compare these two oxazaphosphorines. Recently, the European Organization for Research and Treatment of Cancer (EORTC) presented data that compared single-agent cyclophosphamide (1.5 g/m²) to ifosfamide (5.0 g/m²) with mesna in 123 evaluable patients with adult soft-tissue sarcoma.[30] Although a higher response rate for ifosfamide was observed in all evaluable patients (14.5% v 6.6%) as well as in previously untreat-

Table 4. High-Dose Chemotherapy and Autologous Marrow Transplantation in Refractory Testicular Cancer

Author	No. Patients	Chemotherapy Regimen	No. CR (%)	MDR (wk)
Blijham et al[23]	13	Cy/VP-16	4 (30)	15
Corringham et al[24]	1	melphalan	0	—
Wolff et al[25]	11	VP-16	2 (20)	20, 24
Robinson et al[26]	2	MTX/HN_2/DOX VCR/VBL/MITH/6-TG ACT/5-FU/Cy BCNU/ara-C/CDDP	0	

Abbreviations: MDR, mean duration of remission; Cy, cyclophosphamide; MTX, methotrexate; DOX, doxorubicin; VCR, vincristine; VBL, vinblastine; MITH, mithramycin; HN_2, nitrogen mustard; ACT, actinomycin D; 5-FU, 5-fluorouracil; BCNU, bis-chloroethylnitrosourea; ara-C, cytosine arabinoside; CDDP, cisplatin; 6-TG, 6-thioguanine.

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ed patients (21% v 12%), these differences did not reach statistical significance. Myelosuppression, however, was significantly less for patients treated with ifosfamide. This relative lack of myelosuppression for ifosfamide has appeal for use in combination with other active agents in this disease.

Ifosfamide combination chemotherapy has been recently used for the treatment of testicular cancer by several European investigators. Aigner et al used cisplatin plus ifosfamide in nine patients and achieved a CR in three of these patients.[31] Scheulen and his colleagues combined ifosfamide plus VP-16 in 60 patients and obtained a remission in 24 (40%) of the patients.[32] Hartenstein et al used cisplatin plus ifosfamide plus vinblastine as salvage therapy for patients failing to achieve a CR with PVB induction chemotherapy.[33] Seven CRs and three PRs were obtained in 12 such patients with a mean survival time of 9+ months (range, 4 to 20+ months) for complete responders. Additionally, Bremer and his colleagues used ifosfamide plus VP-16 in the treatment of 36 previously treated patients.[34] Three of 21 patients with unresectable PR and one of 15 patients with progressive disease attained a CR with this combination regimen. Although all these data fail to give specifics regarding response and response duration previous to therapy, it does suggest that an ifosfamide combination regimen is indeed feasible with acceptable toxicity.

The ultimate role for ifosfamide in the treatment of testicular cancer remains to be determined. Because of its activity in highly refractory patients, we feel it is warranted to investigate ifosfamide combination chemotherapy as initial salvage therapy in testicular cancer. We are currently conducting a confirmatory trial at Indiana University using cisplatin plus ifosfamide plus either VP-16 or vinblastine (depending upon prior exposure to drugs), with the substitution of mesna as a continuous infusion in place of NAC. This latter drug will be used because of its lack of gastrointestinal toxicity to determine whether or not we can minimize the nephrotoxicity that was seen in this study.[35]

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ADDENDUM

From group A, patients no. 1, 3, and 5 remain free of disease at 31+, 16+, and 29+ months from start of therapy. Similarly, from groups B and C, patients no. 9, 12, 13, and 16 remain free of disease at 16+, 20+, 15+, and 29+ months from start of therapy.

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Ifosfamide in Refractory Male Germ Cell Tumors

By Benton M. Wheeler, Patrick J. Loehrer, Stephen D. Williams, and Lawrence H. Einhorn

We conducted a phase II clinical trial using ifosfamide (IFX) as a single agent in cisplatin-refractory male germ cell tumor. Thirty patients with measurable disease were treated with IFX, 2 g/m^2 intravenously (IV) for 5 consecutive days every 3 weeks. N-acetylcysteine, 2 g orally every 4 hours, was given as a uroprotective agent. All patients had previously been treated with cisplatin, vinblastine, and bleomycin, and all except two also had previously received etoposide. There were six partial responses (PR) and one complete response (CR) for an overall objective response rate of 23%. The median duration of response was 3.5 months (range, 2 to 5.5 months). The median survival time was 3.5 months (range, 2 to 14+ months). The toxicity of the regimen consisted of hematuria (65% of the patients), nausea and vomiting (43%), neutropenia (WBC < 2,000; 52%), thrombocytopenia (platelet count < 50,000; 20%), and nephrotoxicity (12%). Hematuria was dose related, occurring in 48% of courses using 2 g/m^2/d v only 5% of courses at lower doses. Serious nephrotoxicity (creatinine level > 6.0 mg/mL) was observed in three patients with an elevated pretreatment serum creatinine level. In conclusion, IFX is an active agent in this heavily pretreated population with advanced refractory germ cell tumor. *J Clin Oncol 4:28–34. © 1986 by American Society of Clinical Oncology.*

GERM CELL TUMORS (GCT) represent an important disease of young men. A total of 5,600 new cases are estimated to occur in 1984.[1] With the advent of modern cisplatin-based combination chemotherapy and aggressive surgery, virtually all patients with early-stage disease and 70% of patients with disseminated disease can expect to be cured.[2,3] Despite these excellent results, there remains a patient population not curable with presently available chemotherapeutic agents. Clearly a need exists for new effective drugs in refractory GCT.

Ifosfamide (IFX) is a structural analogue of cyclophosphamide, first synthesized in 1965, in which one chloroethyl group is present on each nitrogen atom. The metabolic transformations of IFX and cyclophosphamide are similar only at differing rates owing to a twofold greater affinity for cyclophosphamide by hepatic activating enzymes.[4] Interest in the use of IFX partially derives from its relative lack of myelosuppression, making it convenient for inclusion into combination chemotherapy. The dose-limiting toxicity of IFX in phase I trials was urothelial damage,[5,6] thought to be due to the production of acrolein, a by-product in the metabolism of IFX.[7] Hemorrhagic cystitis was significantly ameliorated using daily fractionated dosages instead of a single bolus administration,[8,9] and by the use of uroprotective agents such as N-acetylcysteine (NAC)[10,11] or sodium 2-mercaptoethane sulfonate (mesnum).[12,13]

Early clinical trials using IFX showed significant activity in both experimental tumor systems and in human tumors.[14,15] Based upon several European studies suggesting activity of IFX in testicular cancer,[16-21] we initiated a phase II clinical trial using IFX as a single agent in cisplatin-refractory male GCT. This report details our findings.

MATERIALS AND METHODS

Eligibility requirements included a confirmed diagnosis of progressive GCT with bidimensionally and/or serologically measurable disease. All patients must have received prior treatment with cisplatin, vinblastine, bleomycin, and etoposide (VP-16), and have been clearly not curable with further cisplatin combination chemotherapy. All histologic subtypes of GCT were eligible. Patients who progressed during initial treatment with cisplatin, vinblastine, and bleomycin (PVB) but who had

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From the Department of Medicine, Division of Hematology/Oncology, Indiana University School of Medicine, Indianapolis.

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P.J.L. is an American Cancer Society Junior Faculty Fellow; L.H.E. is an American Cancer Society Clinical Professor of Oncology.

Address reprint requests to Lawrence H. Einhorn, MD, Indiana University School of Medicine, Department of Medicine, Division of Hematology/Oncology, University Hospital, Room N-582, 926 West Michigan St, Indianapolis, IN 46223.

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not received VP-16 were also eligible. Additional requirements included a serum creatinine level of < 3.0 mg/dL or a creatinine clearance of 50 mL/min or greater. No patient was excluded because of poor performance status. The protocol was approved by the Indiana University Human Subjects Committee, and informed consent was obtained.

Pretreatment evaluation included full clinical examination, routine laboratory studies (including complete blood cell count [CBC], creatinine level determination, and urinalysis), chest radiograph, and serum β-subunit human chorionic gonadotrophin (HCG), and α-fetoprotein (AFP) level determinations by radioimmunoessay, creatinine clearance, and tumor measurements. These studies were repeated before each course of IFX. Other studies such as computerized tomography (CT) were performed only if necessary for tumor measurements.

The treatment regimen consisted of 2 $g/m^2/d$ IFX in 500 mL 5% dextrose infused intravenously (IV) for a period of 30 minutes for 5 consecutive days, with 2 g (four 500-mg capsules) NAC (Mead Johnson Pharmaceuticals Division, Evansville, Ind), a urothelial protective agent, given orally every four hours beginning at least one hour before and for 48 hours following IFX therapy. Patients were hydrated with 1 L 0.9% saline for a period of four hours before IFX administration and at 100 mL/h continuously throughout the course of therapy. Frequent voiding, at least every two hours while awake and at least once per night, was required. Daily CBC, creatinine values, and urinalysis were obtained during treatment.

The occurrence of hematuria required drug dosage modifications. Hematuria was graded as follows: grade 1, four to ten RBC per high-power field (HPF); grade 2, 11 to 50 RBC per HPF; and grade 3, > 50 RBC per HPF. Grade 1 hematuria did not require cessation of IFX administration. Grade 2 or 3 hematuria was treated by withholding IFX and increasing the dosage of NAC (to 2.5 or 3.0 g every four hours for grade 2 or 3 hematuria, respectively) until resolved. IFX therapy was then either continued at the same dosage or decreased by 25% or 50%.

Serious nephrotoxicity (a creatinine level > 6.0 mg/mL) was encountered early in the study in two patients who had a pretreatment creatinine level > 2.0 mg/dL. Subsequently, the maximum initial dose of IFX was modified based on the serum creatinine level, with lower initial doses being given for a creatinine level above 1.5 Specifically, for creatinine levels < 1.5, the starting dose of IFX was 2.0 $g/m^2/d$; for a creatinine level between 1.5 and 2.0, IFX was given at 1.5 $g/m^2/d$; and for a creatinine level > 2.0, the dosage used was 1.0 $g/m^2/d$.

Courses were given every 3 weeks until progression or until a maximum of six courses had been reached. The dosage of IFX and/or NAC was modified in subsequent courses for hematuria, altered renal function, or myelosuppression.

A complete response (CR) was defined as the complete disappearance of all objective evidence of disease (clinical, radiologic, and serologic). A partial response (PR) was defined as a decrease of 50% or more in the sum of products of perpendicular diameters of measurable disease for at least 1 month. If elevated markers were present as the only evidence of disease, a decrease of 90% or more was required for a PR. Survival times and response durations were measured from the first day of treatment.

RESULTS

Patient characteristics are given in Table 1. Thirty patients were entered in the study between July 1, 1982, and Sept 30, 1983. The median age was 28 years and the median Karnofsky performance score was 80. Twenty-three patients had tumor originating in the testis, whereas seven had an extragonadal primary. Serum markers were elevated in 29 patients.

The extent of disease, as defined by Samuels' criteria,[22] reveals that 73% of the patients had advanced disease at the initiation of IFX therapy. Of the nine patients with advanced abdominal disease, seven had liver metastases. Six patients had concomitant CNS metastases. Three patients had elevated serum markers as their only evidence of disease.

Twenty-two patients had embryonal carcinoma, either alone (11 patients) or with teratoma (11 patients). Three patients had seminoma and one had choriocarcinoma. Four patients with a clinical presentation compatible with GCT, elevated serum markers, and prompt response to an initial cisplatin-containing combination chemotherapy regimen were also included. Three of these patients had extragonadal GCT, two of whom had massively elevated HCG (> 200,000) and probably had choriocarcinoma. Another of these patients had a needle biopsy showing nonseminomatous GCT but it was insufficient for histologic subtyping.

All patients had received prior cisplatin-based combination chemotherapy. Twenty-eight patients had been treated with two or more different cisplatin-containing regimens. Most often these regimens consisted of PVB with or without doxorubicin, followed by cisplatin and VP-16

Table 1. Patient Characteristics

Number	30	
Age (median)	19–42 years (28)	
KPS (median)	30–100 (80)	
Primary site		
Testis	23	
Extragonadal	7	
Extent of disease*		
Minimal pulmonary	4	
Advanced pulmonary	13	22 (73%)
Minimal abdominal ± pulmonary	1	
Advanced abdominal	9	
Elevated marker only	3	

*Samuels' criteria.[22]

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with or without bleomycin. Two patients who progressed during or immediately following initial PVB treatment received only one cisplatin regimen. Fourteen patients had received other additional chemotherapeutic agents, including seven who had been treated with cyclophosphamide. Six patients had also received radiotherapy.

All patients demonstrated incurability with cisplatin-containing regimens. Two patients had incomplete prior treatment records, although both had received PVB and VP-16. Of 28 patients with complete prior treatment records, 21 (75%) progressed during or within 1 month of completion of a cisplatin combination regimen. The other patients also displayed resistance to cisplatin, having progressed within 2 to 3 months of their last course of salvage cisplatin therapy. Twenty-six patients with complete prior treatment records had received VP-16. Of these, 16 had progressed during or immediately following VP-16 therapy and were clearly refractory to this agent, whereas the remainder showed resistance to VP-16, having progressed within 2 to 5 months of their last course of VP-16.

A total of 66 courses of IFX were given. The median number of courses was two (range one fifth to six). Eight courses were not completed because of hematuria followed by progressive disease (seven patients) or death without hematuria (one patient). IFX therapy was started in twenty-four patients at a dosage of 2 $g/m^2/d$. Six patients started at lower dosages because of pretreatment serum creatinine levels between 1.5 and 2.0 mg/dL; five began at 1.5, and one at 1.25 $g/m^2/d$.

All patients were evaluable for response (Table 2). There was one CR and six PRs for an overall objective response rate of 23% (95% confidence intervals, .08 to .38). The median duration of response was 3.5 months (range 2 to 5.5 months). The overall median survival time (MST) was 3.5 months (range, 3 days to 14+ months). The MST for responders was 7 months (range, 2 to 13 months). Only one patient remains alive with progressive disease at 14 months.

Table 2. Response to Therapy

Response	No. of Patients (%)	
CR	1 (3.3)	overall 23% response rate
PR	6 (20)	
None	18 (60)	
Early death	5 (16.7)	
Total	30	

Median duration of response (range), 3.5 mo (2 to 5.5)
Median survival time (range), 3.5 mo (2 to 14+)

Five patients were unable to complete one full course of IFX because of hematuria (four) or death (one). All five had extensive systemic disease with severe dyspnea and massive pulmonary involvement at the onset of IFX therapy; three patients also had liver metastases, and three had CNS involvement. All died of progressive disease 3 to 12 days after the initiation of IFX.

Responses were seen in patients with abdominal disease (three) and pulmonary nodules (three). One patient with an elevated HCG level as his only measurable disease had a 90% decrease of this marker. Six of the seven responders had elevated serum markers; all six had a 90% or greater reduction in abnormal markers (to normal levels in four patients). The one CR had resolution of pulmonary nodules and an elevated HCG marker.

Of the seven responders, six had a testicular primary, and one had a primary retroperitoneal GCT. Six responders had embryonal carcinoma (alone in one and with teratoma in five), and one had seminoma. Three responders were truly refractory to cisplatin and two to VP-16, having progressed during or within 1 month of treatment with these agents. Of the seven patients who had received prior cyclophosphamide, one responded to IFX therapy. This patient had been treated with cyclophosphamide as part of his initial chemotherapy using the Memorial regimen, VAB-6[23]; residual carcinoma was still present after completing this regimen.

TOXICITY

The toxicity of the regimen consisted of hematuria, nausea and vomiting, myelosuppression, and nephrotoxicity (Table 3). All patients were evaluable for hematuria and nausea and vomiting. Other toxicities exclude the five patients with early death.

Overall, 19 patients (65%) experienced hematuria during at least one course of IFX, which was often associated with dysuria and increased urinary frequency. This included one pa-

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Table 3. Toxicity

Characteristic	No. of Patients (%)
Hematuria	19 (63)
Nausea or vomiting	13 (43)
Myelosuppression	
Neutropenia (WBC < 2,000)	13 (52)
Thrombocytopenia (platelets < 50,000)	5 (20)
Nephrotoxicity (creatinine level > 3.0)	3 (12)
Drug death	1 (3)

All patients were evaluable for hematuria and nausea. Other toxicities exclude the five patients with early death.

tient with bilateral nephrostomy tubes and one patient who received only one fourth the recommended dose of NAC. Hematuria occurred during 22 of the 64 courses of IFX (two courses with unclear dosages were excluded) and was clearly dose related (Table 4). Twenty-one of the 44 courses (48%) using IFX at 2 g/m²/d produced hematuria, whereas only one episode of hematuria occurred in 20 courses (5%) using lower doses. Hematuria was frequently severe, being grade 1 in one course, grade 2 in six, and grade 3 in 15 (ten with gross hematuria).

Twenty further courses of IFX were given to eight patients following an earlier episode of hematuria. In 17, hematuria did not recur, responding to increased doses of NAC (seven courses), decreased dosages of IFX (two courses), both increased NAC and decreased IFX (six courses), or no change in either NAC or IFX (two courses). IFX treatment was continued at the same dose (four patients) or decreased 25% (three patients) or 50% (one patient). In one patient hematuria was controlled by increasing NAC administration to 3 g every four hours and decreasing IFX from 2.0 to 1.5 g/m²/d. The IFX dosage was then increased to 1.75 and 2.0 g/m²/d in two subsequent courses, respectively, without recurrence of hematuria. Increased NAC alone failed to control hematuria in three subsequent courses (two patients). One of these patients did not receive another course of IFX. In the other patient, the IFX dosage was later decreased from 2.0 to 1.5 g/m²/d without further hematuria.

Serious nephrotoxicity (creatinine level > 6.0 mg/mL) was observed in three patients, two of whom had a pretreatment creatinine level of 2.0 mg/dL or above. The third patient had a pretreatment creatinine level of 1.9 mg/dL but also received IFX at 2 g/m²/d. Two of these patients required short-term hemodialysis. One patient had had a prior nephrectomy and a creatinine value of 3.4 when starting therapy. Severe azotemia, neutropenia, and thrombocytopenia developed, and he died 2 months after IFX therapy of CNS hemorrhage. This was the only drug-related death. Interestingly, the only measurable disease in this patient was an HCG level of 5,060, which became normal shortly before his death. No autopsy was permitted. No episodes of serious nephrotoxicity were observed in patients treated according to the schedule given in the Methods section.

All patients experienced at least mild nausea with IFX and NAC therapy. Nausea and vomiting was moderate to severe in 13 patients (43%), requiring multiagent antiemetic therapy. This appeared to be temporally related to the administration of NAC in several patients. Repeat administration of NAC was required frequently after emesis of NAC capsules. Inability to consume oral NAC may have contributed to the incidence of hematuria. Nausea and vomiting did not preclude administering further IFX to any patient.

Myelosuppression was significant but manageable. Neutropenia (nadir WBC < 2,000) occurred in 13 patients (52%) and was severe (nadir WBC < 1,000) in eight (27%). Three episodes of granulocytopenic fever occurred, including two with bacteremia. All responded to appropriate antibiotic therapy. Platelet count nadirs < 50,000 were seen in five patients (20%), four of whom had nadirs < 25,000. Mild decreases in the hemoglobin level were common. Moderate (3.0 to 3.9 g) and severe (4.0 to 4.9 g) declines in hemoglobin levels were observed in only one patient each. In our experience, this degree of myelosuppression in such a heavily pretreated patient population was considered moderate in severity.

Table 4. Hematuria

Ifosfamide Dose (g/m²/d)	No. of Courses	No. With Hematuria (%)
2.0	44	21 (48)
1.75	1	0
1.50	16	1 (6)
1.25	1	0
1.0	2	0

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DISCUSSION

IFX has been used extensively in testicular cancer in Europe.[24] Table 5 summarizes the available literature. Details on prior chemotherapy in these studies are generally incomplete. In minimally pretreated patients, the response rate appears to be about 80%, whereas in patients receiving prior cisplatin, the response rate was 20%. In the latter study by Scheulen et al,[21] the pretreatment regimen consisted of vinblastine/bleomycin alternating with cisplatin/doxorubicin. Some patients also later received VP-16. However, the number of patients truly refractory to cisplatin and/or VP-16 is unclear. Studies involving patients not refractory to cisplatin are not strictly comparable with the data presented here. The response rate in our study of heavily pretreated patients with advanced GCT refractory to cisplatin combination chemotherapy was 23%.

Few agents have demonstrated the ability to produce objective responses in patients refractory to cisplatin. VP-16 has shown substantial activity with an overall single-agent response rate of 34% in cisplatin-refractory patients, but durable remissions were not seen.[25] This activity led to its use in salvage regimens that produced long-term survivors in about one third of the patients when combined with cisplatin in patients failing first-line therapy.[26]

More recently, high-dose VP-16 therapy, alone[27] or with high-dose cyclophosphamide[28] and autologous bone marrow transplantation, has been shown to produce response rates of 60% in patients refractory to PVB. Both studies were associated with severe myelosuppression, making them unsuitable for use in combination chemotherapy. Although high-dose cyclophosphamide administration has been shown to have activity in minimally pretreated patients with GCT,[29,30] high-dose cyclophosphamide treatment alone has not been documented to produce responses in patients refractory to cisplatin.

Testicular cancer is a chemosensitive tumor; however, patients with refractory disease progressing on prior cisplatin combination regimens rarely respond to any single-agent or combination chemotherapy regimen. In our experience, we have only rarely seen a PR with active agents from the past such as mithramycin, actinomycin D, doxorubicin, methotrexate, or cyclophosphamide.

IFX is the only single agent other than VP-16 shown capable of inducing an objective response in patients refractory to cisplatin. The overall response rate was 23%, but the response duration was short with no long-term survivors. Although the results appear less impressive than those observed with VP-16, the current patients were much more heavily pretreated, having failed two or more cisplatin-containing regimens, including PVB and VP-16 in all but two patients. Heavy pretreatment may also have contributed to the degree of myelosuppression observed.

Hematuria was a significant problem in this study, resulting in part from the inability to consume effective doses of oral NAC. Hematuria is less frequent in most studies using parenteral uroprotective agents. Mesnum is commonly used in Europe. Boutis et al[19] noted microhematuria in only three of 16 patients (19%) using mesnum. Niederle et al[20] reported only rare failures among 63 patients treated with IFX, VP-16, and mesnum. They noted that myelosuppression became

Table 5. Ifosfamide in Testicular Cancer: European Data

Author	No. of Patients	CR (%)	PR (%)	Responders (%)
Minimal or no prior chemotherapy				
Bruhl et al[16]	43	23 (53)	14 (33)	37 (86)
	9	8 (89)	1 (11)	9 (100)*
Weißach et al[17]	39	8 (21)	14 (36)	22 (56)
Schmoll et al[18]	18	2 (11)	13 (72)	15 (83)
Boutis et al[19]	16	10 (63)	4 (25)	14 (88)
Niederle et al[20]	6	2 (33)	3 (50)	5 (83)*
Total	131	53 (40)	49 (37)	102 (78)
Prior cisplatin				
Scheulen et al[21]	87	1 (1)	16 (18)	17 (20)

*Seminoma.

dose limiting when hematuria was controlled. Mesnum was compared with standard saline hydration and urine alkalinization for uroprotection by Scheulen et al.[21] Hemorrhagic cystitis occurred in 27% of the group given standard uroprotection *v* only 4% using mesnum ($P < .001$) although the mean dose of IFX was higher in the mesnum group (average dose per treatment day of 35 mg/kg/d *v* 48 mg/kg/d, respectively).

The ultimate role for IFX remains to be determined. Since the principal goal of treatment in male GCT is cure, we feel IFX should be studied in second-line salvage regimens. With its different mechanism of action and side effects, it is natural to incorporate IFX into existing salvage regimens including cisplatin and VP-16. The relative lack of myelosuppression of IFX may prove to be a significant advantage. Indeed, several European trials have used IFX in combination chemotherapy approaching the same dosage it was given in single-agent studies.[18,20,31,32] Trials using IFX in salvage combination chemotherapy regimens are currently under way at Indiana University (Indianapolis). Our preliminary results have been very encouraging.[33]

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Ifosfamide
D82-067-825
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July 1986

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PHASE I WORKING GROUP

JANUARY 1986

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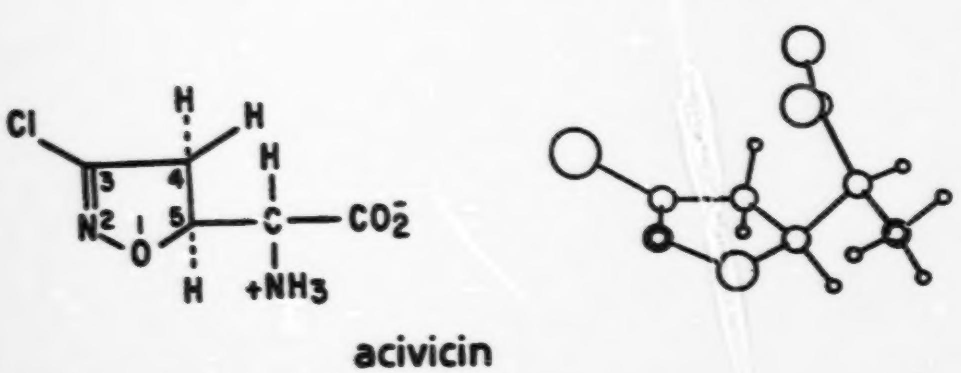
Prevention of Acivicin-Induced CNS Toxicity by Concomitant Amino Acid Infusion. J.P. McGovren and M.G. Williams, The Upjohn Company, Kalamazoo, MI 49001.

Acivicin is an amino acid antitumor agent currently being evaluated in Phase II clinical trials. In humans acivicin causes reversible, dose-limiting CNS effects (somnolence, ataxia, personality changes, hallucinations). We previously showed that acivicin-treated cats exhibit symptoms (ataxia, sedation, somnolence) resembling CNS toxicity reported in humans. We hypothesized that, if acivicin uptake into brain were mediated by a saturable transport system common to endogenous amino acids, we might block drug uptake and CNS toxicity by increasing plasma amino acid levels. Two cats each received constant-rate i.v. infusions at 21 ml/hr of either saline or Aminosyn®, 10% (a mixture of 16 amino acids not containing glutamine, glutamate, aspartate, or cysteine) for 4 hr prior and 18 hr subsequent to treatment with acivicin, 60 mg/kg, i.p. Presence or absence of ataxia and sedation were noted at 18 hr post-acivicin, the time of peak CNS effects in cats. After 3 weeks, the same animals received the alternate (cross-over) treatment. Results: 3/4 saline-infused acivicin-treated cats were ataxic and 4/4 were sedated; 0/4 amino acid-infused cats were ataxic and 0/4 were sedated. In another experiment, cats were infused with saline or the amino acid mixture and treated with acivicin as above, then sacrificed at either 4 or 8 hr post-acivicin and drug levels in brain were quantitated by microbiological assay. Amino acid co-treatment decreased acivicin levels in brain by 75% at 4 hr and 92% at 8 hr compared to saline-infused controls. Groups of mice bearing i.p.-implanted L1210 leukemia were treated i.p. with saline or Aminosyn (0.3 ml/injection) at -15, +15, and +45 min relative to acivicin, 2.5-40 mg/kg/day, daily x 5, i.p. Survival times did not differ significantly between the saline- and amino acid-treated groups. We conclude that co-treatment with an amino acid mixture could protect cancer patients from acivicin-induced CNS toxicity without ablating antitumor efficacy.



acivicin

(αS,5S)-α-amino-3-chloro-2-isoxazoline-5-acetic acid

AT-125, NSC 163501

SLIDE 1. Structure of acivicin, which is a neutral amino acid.

SLIDE 2

CHARACTERISTICS OF ACIVICIN CNS TOXICITY IN HUMANS

- dose-related and reversible; disabling and requiring hospitalization at higher doses in Phase I studies

- severity is schedule-dependent

    - CNS tox>myelotox with single-dose, 24 hr infusion, 72 hr infusion
    - myelotox>CNS tox with daily x5

- symptoms reported in one or more patients from seven Phase I or II studies:

    - disorientation/confusion/sense of unreality
    - lethargy/fatigue/asthenia
    - depression/mood change/anxiety
    - hostility/paranoia/psychosis
    - amnesia/memory loss
    - sleepiness/somnolence/transient unconsciousness
    - lightheadedness/dizziness/subjective unsteadiness
    - dysarthria
    - sleep difficulty/vivid dreams/nightmares

- tremor/focal muscular twitching
- visual, auditory, or olfactory hallucinations
- blurred vision
- headache
- "large thoughts"
- concentration or calculation difficulty
- ataxia/truncal ataxia

- onset: 1-3 days after initiation of therapy

- duration: 3-4 days post-treatment

Cat

- at 60 mg/kg (670 mg/m^2) mydriasis, sedation, ataxia were noted
- at 100 mg/kg (1110 mg/m^2), mydriasis, sedation, somnolence, ataxia, vomiting, and diarrhea were observed
- peak effect: 18 hr; duration: 2 days
- no CNS effects were noted in kittens at doses up to 100 mg/kg

SLIDE 3. Acivicin-treated cats exhibit some CNS symptoms (ataxia, sedation, somnolence), which are consistent with those observed in humans. Rodents do not exhibit detectable CNS toxicity upon acivicin treatment.

Acivicin Effect on Brain Content of Neurotransmitter Amino Acids
(nmoles/mg tissue; mean ± s.d., n = 3 or 4)

Cat

		60 mg/kg, 18 hr		100 mg/kg, 18 hr	
		T	C	T	C
Cerebrum	GLU	12.2 ± 1.1	14.4 ± 0.8	13.4 ± 1.1	13.3 ± 2.2
	GLN	8.5 ± 0.6	9.2 ± 1.3	5.4 ± 0.5	5.7 ± 1.2
	ASP	3.0 ± 0.4	3.5 ± 0.5	3.1 ± 0.3	2.9 ± 0.2
	GABA	2.1 ± 0.2	1.7 ± 0.2	3.2 ± 0.6*	1.5 ± 0.1
Cerebellum	GLU	-	-	11.4 ± 0.8*	13.1 ± 1.0
	GLN	-	-	6.8 ± 0.6*	8.5 ± 0.6
	ASP	-	-	2.1 ± 0.2	2.3 ± 0.1
	GABA	-	-	2.7 ± 0.3*	1.3 ± 0.2
Midbrain	GLU	-	-	8.4 ± 0.8	7.4 ± 0.3
	GLN	-	-	4.5 ± 1.0	4.6 ± 0.7
	ASP	-	-	2.9 ± 1.0	2.6 ± 0.1
	GABA	-	-	3.2 ± 0.8	1.7 ± 1.0

* Significantly different from control (2-sided t-test; $p \leq 0.05$).

SLIDE 4. Cats were treated with acivicin, i.p., at 60 or 100 mg/kg and sacrificed at 18 hr, the time of peak CNS symptoms. Amino acid content of brain was quantitated. Results indicated that GABA was increased ca. 2-fold in cerebrum and cerebellum. Small effects were also seen on glutamate and glutamine content in cerebellum.

SLIDE 5

EFFECT OF AMINO ACID INFUSION ON ACIVICIN-INDUCED BEHAVIOURAL CHANGES IN CATS

- MODEL: 4 CONSCIOUS, UNRESTRAINED FEMALE CATS WITH CHRONICALLY IMPLANTED CATHETERS IN JUGULAR VEIN. TWO CATS RECEIVED ACIVICIN AND AMINO ACID INFUSION, 2 RECEIVED ACIVICIN AND SALINE INFUSION; CROSSED-OVER 3 WK LATER.
- AMINOSYN® 10% (ABBOT) IS A MIXTURE OF 16 AMINO ACIDS (NOT INCLUDING GLUTAMINE, GLUTAMATE, ASPARTATE, CYSTEINE) PLUS ELECTROLYTES. AMINOSYN OR SALINE WAS INFUSED I.V. AT 21 ML/HR FROM -4 TO +18 HR RELATIVE TO ACIVICIN, 60 MG/KG, I.V.
- BEHAVIOUR WAS OBSERVED PRIOR TO STARTING THE SALINE OR AMINOSYN AND AT +1, +14, +18, +24 HR RELATIVE TO ACIVICIN TREATMENT.
- PRESENCE OR ABSENCE OF ATAXIA WAS DETERMINED BY OBSERVING IF CATS COULD WALK ACROSS AN UPENDED WOODEN PALLET WITHOUT FALLING.
- SEDATION AND SOMNOLENCE WERE NOTED BY OBSERVING THE CATS' BEHAVIOUR UPON APPROACH BY EXPERIMENTERS. NORMAL CATS ARE AROUSED EASILY EVEN WHEN ASLEEP AND DISPLAY A NUMBER OF CHARACTERISTIC BEHAVIOURS.

Effect of Saline or Amino Acid Mixture Co-Treatment on Incidence of Toxic Symptoms in Acivicin-Treated Cats (Pooled Cross-Over Data)

	saline					amino acid mixture				
hr post acivicin	pre	1	14	18	24	pre	1	14	18	24
mydriasis	0/4	0/4	4/4	4/4	4/4	0/4	0/4	4/4	4/4	4/4
vomiting	0/4	0/4	3/4	2/4	1/4	0/4	0/4	1/4	1/4	1/4
sedation	0/4	0/4	3/4	2/4	2/4	0/4	0/4	0/4	0/4	0/4
ataxia	0/4	0/4	3/4	3/4	3/4	0/4	0/4	0/4	0/4	0/4

SLIDE 6. Cats were treated with acivicin, 60 mg/kg, i.p., and were infused with either saline or amino acids. Presence or absence of CNS symptoms was noted at intervals after dosing. Results are expressed as the incidence of effects in 4 cats. Aminosyn treatment prevented CNS toxicity completely in all 4 cats studied.

Drug Levels (µg/ml or µg/g) in Plasma and Brain of Cats Treated With Acivicin, 60 mg/kg, i.p.

	saline		Aminosyn, 10%		T/C
	4 hr				
frontal cortex	61.5	57.3	16.2	7.3	
cerebellum	41.8	48.3	17.3	5.9	
diencephalon	85.0	80.0	19.5	7.6	0.25
midbrain	43.3	52.5	21.5	7.7	± 0.06
corpus callosum	34.3	24.3	10.6	7.9	
plasma	55.3	62.7	47.3	36.3	0.71
	8 hr				
frontal cortex	20.5	35.3	3.9	0.9	
cerebellum	19.5	37.3	2.8	1.2	
diencephalon	23.8	25.9	4.2	1.1	0.08
midbrain	22.5	36.3	2.4	1.4	± 0.02
corpus callosum	18.6	27.0	2.5	0.7	
plasma	21.0	31.0	18.6	4.4	0.44

SLIDE 7. Cats were treated with acivicin, 60 mg/kg, i.p. and infused with either saline or amino acids as described in previous slides (2 cats/treatment/time). Cats were sacrificed at +4 or +8 hr after acivicin administration and acivicin levels in various brain regions were quantitated by microbiological assay. Under the "saline" and "Aminosyn, 10%" headings, values for drug levels (µg/ml or µg/g) in plasma or brain of 2 cats are given. "T/C" refers to the mean of levels in plasma or a given brain region of 2 Aminosyn-treated cats divided by the mean of levels in 2 saline-infused cats. The T/C values for all brain regions were averaged and results are expressed as mean ± s.d. Aminosyn co-treatment reduced acivicin levels in brain by 75% at 4 hr. and >90% at 8 hr.

Effect of Co-Treatment with an Amino Acid Mixture on the Antitumor Efficacy of Acivicin in Mice Bearing i.p.-implanted L1210 Leukemia [a]

acivicin dose (mg/kg/day)	day of death (mean ± s.d.; n=6)	
	acivicin + saline	acivicin + amino acid
40	13 ± 1	14 ± 2
20	12 ± 1	12 ± 1
10	11 ± 1	13 ± 3
5	11 ± 2	11 ± 2
2.5	10 ± 1	11 ± 1
control	8 ± 1	8 ± 1

---

[a] Aminosyn®, 10% (0.3 ml/injection) was administered i.p. at -15, +15, and +45 min relative to acivicin, daily x5, i.p.

SLIDE 8. Male B6D2F1 mice (6/group) were implanted with 10^5 L1210 leukemia cells on day 0. Treatment with acivicin and saline or Aminosyn was begun on day 1. Acivicin antitumor efficacy was not affected by Aminosyn co-treatment.

SLIDE 9

Activity of Acivicin in a Human Tumor Cloning System

Total human tumors attempted = 578
Total human tumors evaluable* = 184 (32%)

*≥30 colonies/plate

Data of D. Von Hoff

SLIDE 10

Conditions for Acivicin Testing

1) Two concentrations tested - 0.1 and 1.0 μg/ml
2) Exposure time - 1 hour or continuous

Data of D. Von Hoff

SLIDE 11

In Vitro Activity of Acivicin

Concentration (μg/ml)	Exposure time	# Responses/ # Evaluable	(%)
0.1	1 hr	9/43	(20%)
1.0	1 hr	9/43	(20%)
0.1	Continuous	8/34	(23%)
1.0	Continuous	17/34	(50%)

Data of D. Von Hoff

SLIDE 12

Tumor Specific Activity of Acivicin
(1.0 µg/ml x 1 hr exposure)

Tumor type	# Responses/# Evaluable
Adrenal	0/1
Breast	0/6
Cervix	0/1
Colon	0/1
Corpus uteri	0/1
Gallbladder	0/1
Kidney	0/1
Lung - non small cell	1/3
Melanoma	1/4
Ovary	3/14
Mesothelioma	1/2
Sarcoma	0/1
Stomach	1/1
Thyroid	0/1
Unknown primary	2/5
	9/43 (20%)

Data of D. Von Hoff

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Acivicin
Upjohn
January 1986

SLIDE 13

Tumor Specific Activity of Acivicin
(1.0 μg/ml x continuous exposure)

Tumor type	# Responses/# Evaluable
Adrenal	0/1
Breast	1/3
Cervix	0/1
Colon	0/1
Corpus uteri	0/1
Gallbladder	1/1
Kidney	1/1
Lung - non small cell	3/3
Melanoma	2/4
Ovary	6/13
Sarcoma	1/1
Thyroid	0/1
Unknown primary	2/3
	17/34 (50%)

Data of D. Von Hoff

Conclusions on Acivicin

1) Acivicin is more cytotoxic for human tumor colony forming units when used as a continuous exposure.

2) When used as a continuous exposure at 1.0 μg/ml, Acivicin has a wide spectrum of antitumor activity.

Data of D. Von Hoff

SLIDE 14. If Aminosyn co-treatment in cancer patients prevents CNS toxicity and allows higher doses to be administered safely, antitumor efficacy could be substantially enhanced.

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